Aspects of the selectivity of isoproturon to Bromus sterilis, Bromus willdenowii and barley

Henly, Sarah

Award date:
1986

Awarding institution:
University of Bath

ASPECTS OF THE SELECTIVITY OF ISOPROTURON TO *BROMUS STERILIS*, *BROMUS WILLDENOWII* AND BARLEY

Submitted by SARAH HENLY

for the degree of Doctor of Philosophy

of the University of Bath

1986

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

To my grandfather

CONTENTS

	Page
ACKNOWLEDGEMENTS	vii
ABBREVIATIONS	viii
ABSTRACT	x
1. INTRODUCTION	1
1.1 WEED FLORA	1
1.2 *BROMUS STERILIS*	3
1.2.1 History	3
1.2.2 Description and life cycle	6
1.2.3 Cultural control	8
1.2.4 Chemical control	11
1.3 *BROMUS WILLDENOWII*	17
1.3.1 History	17
1.3.2 Description and life cycle	19
1.3.3 Management	19
1.3.4 Cultural techniques	22
1.3.5 Chemical treatments	22
1.4 ISOPROTURON	24
1.4.1 History	24
1.4.2 Properties	24
1.4.3 Uses	26
1.4.4 Mode of action	27
1.4.5 Metabolism	33
1.4.6 Persistence	37
1.4.7 Environmental factors	38
1.5 OBJECTIVES	41

	Page
2. OPTIMISATION OF EXPERIMENTAL CONDITIONS	43
2.1 INTRODUCTION	43
2.2 MATERIALS AND METHODS	45
2.2.1 Plant production	45
2.2.1.1 Seed supply	45
2.2.1.2 Plant material	45
2.2.1.3 Growth conditions	46
2.2.1.4 Statistical analysis	46
2.2.2 Germination studies	47
2.2.3 Soil type	47
2.2.4 Seed depth	47
2.3 RESULTS	49
2.3.1 The effect of light on seed germination	49
2.3.2 The effect of soil type on seedling emergence and growth	49
2.3.3 The effect of seed depth upon seedling emergence and growth	52
2.4 DISCUSSION	56
3. HERBICIDE APPLICATION	61
3.1 INTRODUCTION	61
3.2 MATERIALS AND METHODS	64
3.2.1 Preparation	64
3.2.1.1 Isoproturon	64
3.2.1.2 Plant material	64
3.2.1.3 Growth substrate	64
3.2.1.4 Growth conditions	65

Page

3.2.2 Persistence of isoproturon 65

3.2.3 Application to seeds 66

3.2.4 Application to soil 66

3.2.5 Application to foliage 67

3.2.6 Timing of treatments 67

3.2.7 Phytotoxicity assessments 70

3.3 RESULTS 71

3.3.1 The persistence of isoproturon in soil 71

3.3.2 The phytotoxicty of isoproturon to seeds 71

3.3.3 The effect of stage of growth on the phytotoxicity of isoproturon following root exposure 74

3.3.4 The effect of stage of growth on the phytotoxicity of isoproturon following foliar application 78

3.4 DISCUSSION 82

4. HERBICIDE RETENTION 91

4.1 INTRODUCTION 91

4.2 MATERIALS AND METHODS 95

4.2.1 Scanning electron microscopy 95

4.2.2 Dye retention 95

4.3 RESULTS 97

4.3.1 Leaf surface morphology 97

4.3.2 Spray retention characteristics 101

4.4 DISCUSSION 103

	Page
5. HERBICIDE UPTAKE	107
5.1 INTRODUCTION	107
5.2 MATERIALS AND METHODS	111
5.2.1 Localised uptake from soil	111
5.2.2 Seed depth effects	112
5.2.3 Uptake from nutrient culture	112
5.2.4 Radioisotope determinations	114
5.2.4.1 Source of activity	114
5.2.4.2 Plant material	115
5.2.4.3 Solution sampling procedure	115
5.2.4.4 Tissue sampling procedure	115
5.2.4.5 Counting technique	117
5.2.4.6 Quench correction	117
5.2.4.7 Evaporation correction	118
5.2.4.8 Weight correction	118
5.3 RESULTS	119
5.3.1 Phytotoxicity following exposure of the sub-surface shoot and roots	119
5.3.2 The effect of seed depth on phytotoxicity following soil surface treatments	119
5.3.3 Phytotoxicity following exposure to isoproturon in nutrient culture	122
5.3.4 The uptake of ^{14}C-isoproturon by whole plants	122
5.4 DISCUSSION	129

Page

6. HERBICIDE TRANSLOCATION 136

6.1 INTRODUCTION 136

6.2 MATERIALS AND METHODS 138

6.2.1 Application of ^{14}C-isoproturon 138

6.2.2 Autoradiography 138

6.2.3 Translocation rates 140

6.3 RESULTS 141

6.3.1 Direction of movement of ^{14}C-isoproturon 141

6.3.2 Rate of movement of ^{14}C-isoproturon 141

6.4 DISCUSSION 149

7. HERBICIDE MODE OF ACTION 152

7.1 INTRODUCTION 152

7.2 MATERIALS AND METHODS 155

7.2.1 Infra-red gas analysis 155

7.2.2 Chlorophyll fluorescence 155

7.2.3 Chloroplast isolation 159

7.2.4 Chlorophyll determination 159

7.2.5 The oxygen electrode 160

7.3 RESULTS 162

7.3.1 The effect of isoproturon on photosynthetic rates 162

7.3.1.1 Carbon dioxide uptake 162

7.3.1.2 Fluorescence yields 166

7.3.2 The effectiveness of *in vitro* chloroplast electron transport inhibition by isoproturon 173

7.4 DISCUSSION 175

	Page
8. HERBICIDE METABOLISM	180
8.1 INTRODUCTION	180
8.2 MATERIALS AND METHODS	182
8.2.1 Thin-layer chromatography	182
8.2.2 Measurement of R_{ip} values	183
8.2.3 Densitometer scanning	183
8.3 RESULTS	184
8.3.1 Isoproturon degradation	184
8.4 DISCUSSION	195
9. GENERAL DISCUSSION	198
10. REFERENCES	205
11. APPENDICES	236

ACKNOWLEDGEMENTS

I wish to express my thanks to both Dr. Alan Dodge and Mr. Dick Stephens for their continual advice and helpful criticism throughout this work. I am indebted to the technical staff at Bath University, especially to Mr Tim Moxham and to Mr Pete Clark; to Mr Dave Hornsey for advice on radioisotopes; to Mr P.K. Nandi for statistical advice; to my colleagues, in particular Dr. Paul Knox and Dr. Deborah Longman for constructive criticism; to my fellow scientists at the former WRO for helpful suggestions; and to my farming cousins at Harnhill for practical advice.

I am grateful to May & Baker AG and Ciba-Geigy AG for the donation of isoproturon and ^{14}C-isoproturon respectively, to the former WRO for seed supplies and to the MAFF for a research studentship.

ABBREVIATIONS

A	absorbance
AAB	Association of Applied Biologists
AFRC	Agriculture and Food Research Council
a.i.	active ingredient
approx.	approximately
BCPC	British Crop Protection Council
Bq	becquerel, 1 Ci = 3.7×10^{10} Bq
°C	degree Celsius
^{14}C	^{14}carbon
Chl	chlorophyll
Ci	Curie
concn	concentration
D/N	day/night
diam.	diameter
df	degrees of freedom
dpm	disintegrations per minute
E	Einstein
EWRC	European Weed Research Council
F	F-distribution
F.C.	field capacity
FW	fresh weight
GS	growth stage
ha	hectare
IRGA	infra-red gas analyser
IUPAC	International Union of Pure and Applied Chemistry
I_{50}	inhibitory potency (50%)

M	molar
MAFF	Ministry of Agriculture, Fisheries and Food
min	minute
ms	mean sum of squares
NIAB	National Institute of Agricultural Botany
NS	not significant
P	probability
Pa	pascal
P_{fr}	phytochrome - far red-absorbing form
P_{r}	phytochrome - red-absorbing form
PS I	photosystem I
PS II	photosystem II
RH	relative humidity
rpm	revolutions per minute
RSC	Royal Society of Chemistry
s; sec	second
SD	standard deviation of series
SE	standard error of mean
SS	sum of squares
TLC	thin-layer chromatography
V	volts
v/v	volume/volume (concn.)
w/v	weight/volume (concn.)
WRO	Weed Research Organisation

ABSTRACT

Various factors which may account for the differential response of *Bromus sterilis*, *Bromus willdenowii* and barley to isoproturon were examined. Isoproturon was applied either as a soil drench or a foliar spray, to controlled environment-grown seedlings at various stages of growth. All three species were at their most sensitive at the 1st leaf stage (Zadoks GS 11), *B. sterilis* showing greatest susceptibility to isoproturon. *B. willdenowii* seeds were the most vulnerable when germinated in isoproturon solution, however seedlings of this species were relatively tolerant in soil tests. Foliar applications were less phytotoxic to all seedlings than were root drenches, which may be related to the poor retention of spray solution on leaf surfaces. Morphological differences between species were not considered to contribute to selectivity, although root size and distribution in relation to isoproturon in the soil was important in determining phytotoxicity. Greater uptake of the herbicide by the *Bromus* spp. was confirmed by inclusion of ^{14}C-isoproturon in nutrient solution containing 2 week old seedlings. The amount of ^{14}C-isoproturon translocated by *B.sterilis* was greater than that by the other species following foliar applications to the 1st leaf of 2 week old plants. Selectivity appeared to be primarily related to rates of uptake, translocation and the efficiency of herbicide metabolism. The role of metabolism was indicated by rates of recovery from photosynthetic inhibition and autoradiography of TLC plates spotted with extracts from ^{14}C-iso proturon-treated seedlings. Rapid uptake and translocation, coupled with slow metabolism rendered *B. sterilis* more susceptible to isoproturon. Poor translocation rates, and a requirement for greater

concentrations of isoproturon to inhibit photosynthetic electron transport (I_{50} value) in *B. willdenowii* conferred greater tolerance to this species. Rapid recovery of barley from photosynthetic inhibition indicated that degradation of isoproturon was most rapid in this species, explaining its tolerance.

1. INTRODUCTION

1.1 WEED FLORA

"A weed is a plant growing in the wrong place".

Since the advent of farming, weeds have interfered with crop production, and their removal has necessitated massive energy inputs. Crop husbandry techniques, including crop rotation, tillage and stubble burning replaced the primitive method of hand-weeding. The use of chemicals for weed control was not recognised until 1896, when Bonnet reported the efficacy of Bordeaux mixture in the control of charlock *(Sinapis arvensis* L.). The first chemicals were non-specific and generally highly phytotoxic. These later gave way to more complex compounds with enhanced selectivity. As a result of the use of herbicides, many changes have occurred in weed flora. Some formerly unimportant weeds have become prominent, while previously important species have been eliminated by chemicals, as illustrated in Figure 1.1 (Fryer and Chancellor, 1970). For example, the introduction of 2,4-D and MCPA in the 1940s resulted in the control of several important broad-leaved weeds of U.K. cereals, namely poppy *(Papaver rhoeas* L.) and charlock. The 'ecological niches' left vacant by these susceptible broad-leaved weeds were filled by the less susceptible cleavers *(Galium aparine* L.) and chickweed *(Stellaria media* (L.) Vill.), which themselves in turn were controlled by newer herbicides such as mecoprop and dichlorprop. More recently, grass weeds such as wild oat *(Avena fatua* L.), blackgrass *(Alopecurus myosuroides* Huds.) and barren brome *(Bromus sterilis* L.) have predominated.

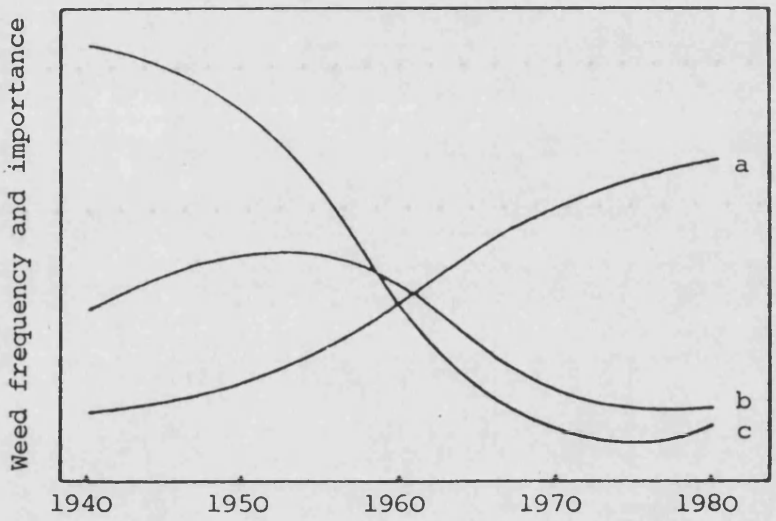


FIGURE 1.1

Conjectured changes in the British arable weed flora over four decades (redrawn from Fryer and Chancellor, 1970). a) Grass weeds

b) Less susceptible broad-leaved weeds

c) Susceptible broad-leaved weeds

During the last decade, many selective herbicides specifically for grass weed control have been produced, and as these problems are reduced, broad-leaved weeds such as cleavers reappear.

Changes in agricultural practice and techniques which alter conditions at the level of the micro-habitat, have also influenced the composition of weed flora (Hammerton, 1968). The introduction of minimal cultivation systems has required a greater dependence upon chemical weed control. In cereals, the present inability to control barren brome selectively poses a serious threat to the continuous use of minimal cultivation. However, a similar threat was presented by couch grass *(Elymus repens* (L).Nevski) before the advent of glyphosate (Froud-Williams *et al.*, 1981). It is hoped that a solution to this particular problem weed will rapidly be found.

1.2 *BROMUS STERILIS*

1.2.1 History

Prior to 1970, *Bromus sterilis* L. (barren or sterile brome) was recognised as a common annual grass of field margins and open hedgerows (Rule, 1981) (Plate 1.2). More recently, it has occurred as a weed of arable crops, especially wheat *(Triticum aestivum* L.) and barley *(Hordeum vulgare* L.) (Froud-Williams *et al.*, 1980) (Plate 1.1). The rapid proliferation of *B. sterilis* has been closely associated with changes in farming methods during the last decade (Ayres and Richardson, 1981). Increased national infestations were initially attributed to the intensification of winter cereal acreage (Froud-Williams *et al.*, 1980). However, while the area of winter cereals increased by 24% over the years

PLATE 1.1

B. sterilis in a crop of winter barley.

PLATE 1.2

B. sterilis as a common grass of field margins.

1977-1979 (Home Grown Cereals Authority, 1980), levels of brome infestation increased by 40%. In addition to monocropping, the trend towards minimum cultivation probably accounts for the observed population explosion (Gray, 1981). Direct drilling, following minimal soil disturbance, provides environments suitable for seed germination (Pollard, 1982). The weed seeds that germinate after drilling would previously have been buried by ploughing.

Severe infestations (500 plants m^{-2}) can reduce cereal yields by up to 45% (Gray, 1981), and reduce the quality and therefore the value of harvested grain. *B. sterilis* has a fast growth rate (Grime and Hunt, 1975), which explains its highly competitive behaviour in cereal crops. In 1981, the weed was observed in 9% of winter cereals (Froud-Williams and Chancellor, 1982). The following year, this figure had risen to 15%, making *B. sterilis* the fifth most frequent grass weed in winter cereals (Chancellor and Froud-Williams, 1984). This weed was more competitive towards wheat (12% of fields surveyed in 1981) than barley (4% of fields) (Cousens *et al.*, 1985).

Reports of infestations in spring cereals are not as common as in winter cereals (Budd, 1981), since seeds of *B. sterilis* are shed just prior to autumn sowing. Autumn germinating seedlings of *B. sterilis* compete with the crop whilst those germinating in the spring produce ripe seeds in August when the crop is being harvested, thus contaminating the grain. Contamination ranges from severe (11%) (Froud-Williams, 1983) to slight (0.4 - 1.1% in winter barley) (Budd, 1981). More than three *B. sterilis* seeds per 100 g is considered as serious contamination of barley grain (Mock

and Amor, 1982). Use of non-certified farm saved cereal seed creates a high risk of spreading *B. sterilis* (Budd, 1981).

The success of this weed is partly due to its ability to germinate in a wide range of soil types, though heavy infestations generally only occur on calcareous clay soils overlying chalk or limestone (Froud-Williams, 1982). In the U.K., *B. sterilis* is a particularly serious problem in parts of the East Midlands, especially Bedfordshire and Cambridgeshire (Froud-Williams and Chancellor, 1982), and Southern England where this type of soil predominates. It is well established in most European countries and in parts of the U.S.A.

B. sterilis is also a problem in herbage seed (Budd, 1981) and broad-leaved crops in the Midlands and South-West England. Winter oilseed rape *(Brassica napus* L.) and field beans *(Phaseolus vulgaris* L.) are the most commonly infested broad-leaved species.

1.2.2 Description and life cycle

Seedlings of *B. sterilis* are green or purplish with softly hairy leaf blades and sheaths, the upper surfaces being prominently ribbed (Hubbard, 1968). The mature plant may have as many as ten fertile tillers, supporting drooping purple-coloured panicles. Each panicle can produce up to ten spikelets, each with 4 - 10 florets. The seeds are typically long (approx. 1 cm) and narrow (approx. 1 mm) with awns of up to 3 cm. Each plant produces about 200 seeds, with an average seed weight of 8 - 10 mg (Froud-Williams *et al.*, 1980).

Most of the seeds are shed from late June to early August (Froud-Williams *et al.*, 1980). Germination proceeds rapidly since they have almost no innate dormancy (Froud-Williams, 1981). Harper (1959) defined dormancy of three types, namely innate, enforced or induced. Enforced dormancy he described as an inability to germinate due to an environmental restraint, whereas induced dormancy is an acquired condition caused by some experience after ripening. Seeds showing induced dormancy fail to germinate on return to favourable conditions. Dormancy of *B. sterilis* seeds can be enforced by temperatures of below 2°C, or by drought as in the autumn of 1978 (Froud-Williams *et al.*, 1980). However, Grime (1979) suggested that *Bromus* spp. exploit gaps in vegetation caused by drought. *B. sterilis* seeds are seasonally adapted to the onset of rain following summer drought (Froud-Williams, 1983). It is generally assumed that the recent increase in the incidence of *B. sterilis* is a consequence of the drought experienced in 1976 (Froud-Williams, 1981). Ripe seeds that are shed early in the season (June) sometimes show rudimentary dormancy (Froud-Williams *et al.*, 1980). This has been attributed to inhibition by sunlight, an unusual response of weed seeds. Germination of most arable weed seeds is stimulated by light and inhibited by darkness or far-red light (Toole, 1973) but certain grasses germinate well in the dark (Grime and Jarvis, 1975). Germination of freshly-shed *B. sterilis* seeds is significantly delayed with low intensity red light (Froud-Williams, 1981) or light of a particular quality (Pollard, 1982). This was also reported earlier for freshly collected seeds of *B. tectorum* (Hulbert, 1955).

Light enforced dormancy is a response resulting from photo-inhibition of germination by P_{fr} (the active form of phytochrome) (Hilton, 1982). P_r (inactive form) is converted to P_{fr} when stimulated by red light and reversed when stimulated by far-red or darkness. *B. sterilis* seeds contain very little P_{fr} and do not produce P_{fr} in darkness, and therefore will germinate well in the dark. In dry seeds pre-treated to have high levels of P_{fr}, far-red irradiation promoted germination in *B. sterilis* but red irradiation totally reversed the effect (Bartley and Frankland, 1984). In natural sunlight, which contains red light, P_r is converted to P_{fr}, which is itself actually inhibitory to germination (Hilton, 1982; 1984). Thus *B. sterilis* seeds germinate well in the dark and in far-red light, which is characteristic of shady field margins (Grime and Jarvis, 1975).

Where dormancy is enforced by environmental factors, seeds of *B. sterilis* can persist in the soil until the following spring. Delayed shedding of the basal seeds in each spikelet from the parent plants also extends the period over which germination can proceed (Froud-Williams *et al.*, 1980). *B. sterilis* seeds do not generally remain viable for longer than one year in the soil, forming only a transient seed bank (Thompson and Grime, 1979). Viability of seeds on the soil surface lasted only 9 months (Froud-Williams, 1983), though Gray (1981) recorded germination after 14 months in uncultivated soil.

1.2.3 Cultural control

Cultural control of *B. sterilis* is relatively effective in its objectives of firstly, preventing new seed

entering the field, and secondly, breaking the life cycle of existing populations (Froud-Williams *et al.*, 1980). Any break in the life cycle should, in theory, eradicate the weed, since it lacks a persistent seed bank. Control measures imposed between harvest and sowing of the cereal crop are the most practical.

Prevention of spread of *B. sterilis* seeds is as important as removing the existing weeds. The maintenance of a weed-free headland strip or firebreak around the fields infested with *B.sterilis* to some extent limits weed spread (Anon, 1981). These require constant upkeep by either cultural or herbicidal treatment, and are therefore financially unacceptable to some farmers (Froud-Williams *et al.*, 1980). Froud-Williams *et al.* (1980) reported that spraying hedge bottoms to remove *B. sterilis* was inadvisable, since bare patches are then left available for colonisation. Budd (1981) stressed the need for thorough seed cleaning in areas where *B. sterilis* has been found.

The advantage of the absence of seed dormancy in *B. sterilis* becomes apparent when control is necessary. The majority of seeds germinate soon after dispersal (Froud-Williams, 1981), which corresponds with autumn sowing of the crop. Deep cultivation, burying *B. sterilis* seeds, results in poor seedling emergence (Rule, 1981), and is the single most effective measure of cultural control (Gray, 1981). The importance of soil inversion to a depth of at least 12.5 cm is stressed (Gray, 1981), but it is sometimes difficult to achieve this on the shallow, stony soils where *B. sterilis* predominates. Effective control has been reported using the mouldboard plough after harvest (Runyan and Peeper, 1978; Anon, 1980; Budd, 1981). Buried seed will germinate but fail

to emerge (Froud-Williams, 1983). Shallow cultivation, while less effective than ploughing, can reduce the seed population by 34% (Froud-Williams, 1983), however the recent change to minimum cultivation practices with much reduced soil disturbance creates conditions in which *B. sterilis* thrives. Under minimum cultivation, it has been found that a tined drill gives better control than a single disc direct drill, because of greater soil disturbance with this type of drill (Rule, 1981).

Crop sowing dates influence the degree of infestation with *B. sterilis* (Rule, 1981). Delayed sowing encourages populations of *B. sterilis* to germinate in bare soil before the crop is introduced (Froud-Williams *et al.*, 1980). The stale seedbed technique can be employed if sufficient time is available between harvesting and sowing of the autumn crop (Gray, 1981). Cultivation of the upper 2 - 4 cm will produce a fine tilth for germination of the newly shed seeds of *B. sterilis* so that the germinating seedlings can then be destroyed by a non-selective herbicide such as paraquat. However, farmers wish to sow their crops early in the autumn because early sowing generally results in higher yields. Avoidance of late sowing is justifiable, but Jarvis (1981) pointed out a failure to appreciate that early sowing may lead to increased weed and disease problems.

Efficient stubble burning reduces seedling numbers and destroys surface seed populations of *B. sterilis*, and other annual weeds such as wild oat (Wilson and Cussans, 1975) and blackgrass (Moss, 1979). Straw burning destroyed 97% of ungerminated seed on the soil surface and reduced seedling numbers of *B. sterilis* by 94% (Froud-Williams, 1983). However, seedlings which did survive

were highly prolific and fecund.

Crop rotation, or the inclusion of a spring-sown crop, e.g. spring barley, may be the only effective means of controlling *B. sterilis* culturally (Froud-Williams *et al.*, 1980) in areas where minimum cultivations are practised. The most common break crops used for this purpose are winter oilseed rape and sugar beet (*Beta vulgaris* L.). Herbicides such as propyzamide and ethofumesate ~~simazine~~ respectively will eradicate *B. sterilis* without damaging these crops (Anon, 1980).

Increasing the density of a crop reduces crop yield losses to *B. sterilis* and limits the number of weed seeds produced (Gray, 1981). Other evasive tactics include the use of taller varieties of cereals which are more competitive to *B. sterilis*. Regular cutting in hedge banks reduces the competitive ability of *B. sterilis* (Gray, 1981).

Therefore, traditional methods of cultivation such as ploughing are capable of controlling *B. sterilis* in many cases, yet this method has often been superseded by direct drilling practices. It may be necessary to utilise herbicides in conjunction with cultural techniques to contain this weed.

1.2.4 Chemical control

When *B. sterilis* became a locally damaging weed in cereals, several herbicides noted for their ability to control annual grass weeds were re-examined.

Control of *B. sterilis* in arable crops such as oilseed rape and sugar beet poses no serious problem, since the herbicides propyzamide and ethofumesate ~~simazine~~ are selective within these crops

respectively (Anon, 1980). Ethofumesate destroys *B. sterilis* seedlings in grass seed crops such as perennial ryegrass *(Lolium perenne* L.) (Froud-Williams *et al.*, 1980).

Although various treatments are available for control of *B sterilis* in other crops, none of them are considered safe for use in cereals (Rademacher, 1959; Deloraine *et al.*, 1965). Unfortunately it was found that *B. sterilis* is resistant to many of the herbicides currently used in winter cereals (Froud-Williams *et al.*, 1980; Ayres and Richardson, 1981). As a group, the substituted urea herbicides were found to have the greatest effect on *B. sterilis* when used selectively in wheat and barley (Pollard and Richardson, 1981). However, chemical control in cereals is a major problem due to the variable response of this weed to herbicides (Ayres and Richardson, 1981; Orson, 1981; Pollard and Richardson, 1981). No consistent level of control can be obtained from any single herbicide, even in controlled environments (Froud-Williams *et al.*, 1980). The reasons for this response are not at all clear (Orson, 1981). Varying environmental conditions, such as soil and weather fluctuations, could not account for the apparent variability in the several substituted urea herbicides tested (Ayres and Richardson, 1981).

Despite this variability, several herbicides have exhibited good phytotoxic activity in *B. sterilis* infested fields. The timing of herbicide application may influence its effect upon the weed (Roberts, 1982). A specific chemical may have use in pre-emergence but not post-emergence control of *B. sterilis* or *vice versa* (Ayres and Richardson, 1981).

Prior to drilling of the crop, young seedlings of *B. sterilis* can be controlled with small doses of a contact herbicide such as paraquat (Whybrew, 1969). Any non-selective herbicide treatment which kills the vegetation quickly without leaving a phytotoxic residue in the soil is equally desirable for this purpose (Roberts, 1982). However, once the crop is sown, pre-emergence control of *B. sterilis* requires a selective compound, which should have residual properties if it is to kill weed seedlings as they germinate over an extended period.

Emergence of *B. sterilis* seedlings is prevented by incorporating triallate into the soil (Anon, 1980). An emulsifiable concentrate formulation of triallate gave better results than the granular formulation. A triallate/metoxuron sequence gave 95% control of *B. sterilis* in winter wheat (Jarvis, 1982) and triallate/EPTC incorporated gave good pre-emergence control in the field (Ayres and Richardson, 1981). These field observations have been substantiated by experiments carried out in pots under glasshouse conditions. Ayres and Richardson (1981) recorded that triallate, at a rate of 2 kg a.i.ha^{-1}, shows an appreciable effect upon *B. sterilis*, giving 96% control. Useful control at the pre-emergence stage of *B. sterilis* was also obtained using nitrofen and perfluidone. Nitrofen, at a rate of 2 kg a.i.ha^{-1}, gave 90% control of *B. sterilis* in pot experiments. Perfluidone had potential for use at direct drilling in wettable powder formulation, since it exhibited 99% control at 1 kg a.i.ha^{-1} (Pollard and Richardson, 1981). Of the substituted urea herbicides tested, only isoproturon was adequately selective for *B. sterilis* control at the pre-emergence stage. They suggested,

from pot trial results, that the threshold rate for selective control in cereals is 0.5 kg a.i.ha^{-1}, any larger amount damaging the crop. The recommended rate for pre-emergence applications of isoproturon for control of other grass weeds in the field is 2.5 kg a.i.ha^{-1} (Fryer and Makepeace, 1978). Field trials with isoproturon have not yet established the most effective rate to apply for pre-emergence *B. sterilis* control. Ayres and Richardson (1981) found that isoproturon is highly active against *B. sterilis*, regardless of the environmental conditions. There are many other herbicides that reduce populations of *B. sterilis*, yet they lack sufficient selectivity in wheat and barley when applied pre-emergence.

Larger plants of *B. sterilis* may survive contact herbicide treatments applied prior to crop drilling, and thus interfere with the emerging crop (Ayres and Richardson, 1981). A selective foliar spray is required to destroy surviving seedlings (Roberts, 1982). *B. sterilis* appears to become more tolerant of herbicides after the 3 leaf stage (Zadoks G.S. 13) (Palmer, 1981; Zadoks *et al.*, 1974). In the field, Palmer (1981) found that for most herbicides, application at the 1 - 3 leaf stage (Zadoks G.S. 11-13) of the weed provided most consistent control. With certain chemicals, control is also much more variable at tillering (Zadoks G.S. 21) of *B. sterilis* than at the 1 - 3 leaf stage (Redbond, 1980). The densely hairy character of the whole leaf surface may result in low penetration or retention of the spray and thus limit the control achieved with post-emergence herbicides (Froud-Williams *et al.*, 1980). The addition of a surfactant to the spray increases retention of liquids which, although it improves control

of *B. sterilis*, also reduces crop tolerance (Pollard and Richardson, 1981).

In pot experiments, post-emergence treatments of metoxuron, isoproturon and terbutryne showed potential for control of *B. sterilis* in cereals (Ayres and Richardson, 1981). Metoxuron at 3 kg a.i.ha^{-1} gave 86% control, but at this rate the margin of selectivity in wheat and barley was low (Pollard and Richardson, 1981). Cutting (1980) reported successful early post-emergence control of *B. sterilis* in the field with metoxuron. Pollard and Richardson (1981) reported good activity of isoproturon in pot trials at the 3 leaf stage, but crop damage resulted from doses of over 1.5 kg a.i.ha^{-1}. Orson (1981) found the lowest number of panicles m^{-2} of *B. sterilis* using isoproturon at the 1 - 3 leaf stage at 2.5 kg a.i.ha^{-1}. There was little or no extra yield response using rates higher than those recommended, though control of the weed was usually higher. Isoproturon was the only herbicide that showed potential for both pre- and post-emergence control of *B. sterilis* in pot experiments (Pollard and Richardson, 1981). Results of pot trials using terbutryne at 2 kg a.i.ha^{-1} suggest that it is a useful herbicide for post-emergence control (Ayres and Richardson, 1981). In the field, terbutryne at this rate arrests the development of *B. sterilis*, however, it also retards crop growth (Pollard and Richardson, 1981).

Asulam at 1.5 kg a.i.ha^{-1} showed a sufficient margin of selectivity between *B. sterilis* and several barley varieties in field trials (Giffard *et al.*, 1983). Most other herbicides tested had limited effects on the density of *B. sterilis*, yet some, for example, chlorsulfuron, stunted its growth and so reduced

competition (Richardson *et al.*, 1981).

Several mixtures had greater effects than products applied alone. Isoproturon/ioxynil/bromoxynil gave better post-emergence control of numbers and bulk of *B. sterilis* in the field than isoproturon alone (Atkin and Foster, 1981), with only transient crop damage (Black and Hewson, 1982). A mixture of metoxuron/ simazine showed an appreciable effect at 3 kg a.i.ha^{-1}, though metoxuron alone gave greater % control in pots (Ayres and Richardson, 1981). In the field, the mixture gave 75% reduction in panicle numbers of *B. sterilis* at 4 kg a.i.ha^{-1}, a better % reduction than for metoxuron alone (Pollard and Richardson, 1981).

The above named herbicides are all useful for application at the 1 - 3 leaf stage of *B. sterilis*. For year round control, it may be necessary to apply chemicals more than once during the crop growing season (Rule, 1981). Anon (1981) recommend an application of triallate granules in the autumn, followed by an early post-emergence spray of metoxuron and a further treatment of metoxuron in the spring. A new recommendation in 1985 for low-cost sequential treatment involved the application of cyanazine pre-emergence followed by a cyanazine/isoproturon mixture (Anon, 1985). There are many other recommended sequences, and these vary with soil type, crop variety and climate. Yield responses from the removal of *B. sterilis* are sufficiently high to justify the application of at least two herbicide treatments (Anon, 1981).

The demand continues for a herbicide which can be applied in the field when *B. sterilis* infestations occur. Increased emphasis is being placed upon studies of herbicide mixtures, since no single herbicide has given more than 75% weed control without

producing symptoms of damage to the crop (Pollard and Richardson, 1981). It is often not possible to achieve a high enough level of control to rely on herbicide alone to combat *B. sterilis* (Orson, 1981). Rule (1981) suggested that a combination of chemical and cultural treatments may be most successful.

1.3 *BROMUS WILLDENOWII*

1.3.1 History

Bromus willdenowii Kunth., (formerly *B. catharticus* Vahl.; ex- *B. unioloides* H.B.K. (rescue grass)), commonly known as prairie grass, is a species of brome that originated in North and South America. As its name implies, it was widely used in grassland farming and was introduced into Australia and New Zealand in the 1960s, intended for grazing on high fertility dairy farms (Rumball, 1974). In New Zealand, it was grown predominantly in mixed swards with perennial ryegrass, cocksfoot *(Dactylis glomerata* L.) and white clover *(Trifolium repens* L.). (Allen *et al.*, 1974; Henderson and Grant, 1974). *B.willdenowii* is very palatable and digestible to grazing animals, but its vulnerability to trampling and its open, upright growth habit make it more suitable for cutting than for direct grazing. Its success as a forage crop has led to its introduction to Europe, especially Western and S.W. France, where it now occupies more than 20,000 ha of farmland (Anon, 1984a). New varieties such as Bellegarde, Luprime and Grasslands Matua are grown in France, mainly for zero-grazing or silage. Since 1979, trials have been undertaken in Britain with *B. willdenowii* by NIAB, and several varieties offer considerable promise for use as forage crops on dry soils.

PLATE 1.3

A mature plant of *B. willdenowii*

PLATE 1.4

B. willdenowii sown in a mixture with lucerne.

1.3.2 Description and life cycle

Seeds of *B. willdenowii* are large (6 mm long and 2 mm wide) with pointed or short-awned (1 to 3 mm long) 9 to 13-nerved lemmas. De-awned seeds (burning process) are available in France, variety Bellegarde. The seedling appears similar to that of *Bromus sterilis* until the 2 - 4 leaf stage, when the leaves become larger, wider and more prominently ribbed. The mature plant is a loosely-tufted, short-lived perennial with strongly compressed 6 to 12-flowered spikelets of 16 to 40 mm long (Hubbard, 1968)(Plate 1.3).

Germination is slow, especially at low temperatures (Culleton and McCarthy, 1983), thus seeds should be sown before late August to mid-September to allow good establishment. The plants cease tillering in the autumn, these older tillers heading in early May, and spring-formed tillers 3 weeks later. A good root structure will develop in the second year of growth, and the plants will generally persist for 3 - 6 years. There is active growth in cool seasons and mid-summer, and the plants have good drought and frost resistance (Betteridge and Baker, 1973). Varieties of *B. willdenowii* readily self-seed, thus developing denser swards.

1.3.3 Management

Grasslands Matua, the only variety currently available in Britain has consistently outyielded perennial ryegrass by around 30% in trials at NIAB (Anon, 1984a) and in France (Simon *et al.*, 1983). In the latter part of the season, growth was almost double that of S24 ryegrass (Figure 1.2).

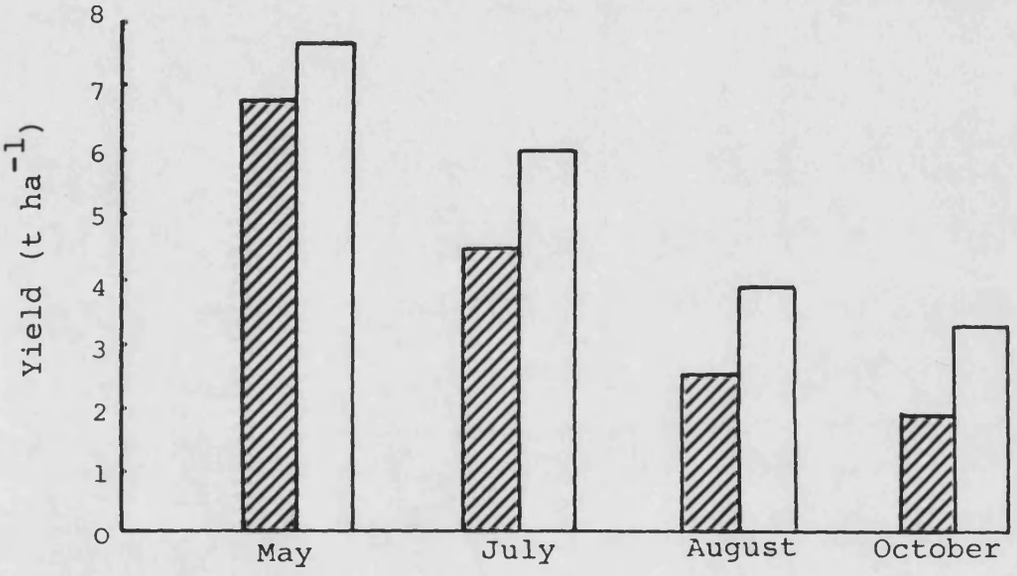


FIGURE 1.2

Yields of *Bromus willdenowii* cv. Grasslands Matua ☐ and *Lolium perenne* cv. S24 ▨ at different times of the year (after Anon, 1984).

Cutting at an optimum height of 5 cm (Etève, 1982) can begin in late May, and continue at 5 week intervals (Parneix, 1982) as with perennial ryegrass. The leaves and stem of *B. willdenowii* have a high sugar content, which obviates the need for silage additives, and the forage produced is highly digestible and palatable. The digestibility of the 1st and 2nd cuts has been comparable with early or intermediate ryegrass, despite the presence of some heads in the crop as early as the first week in May (Etève, 1982).

Machinery wheeling and the trampling from continuous grazing tend to damage the larger tillers that make up older swards (Betin and Mansat, 1979; Peyraud, 1983). Grasslands Matua seems to be more palatable to grazing cattle than ryegrass, but the animals seem to dislike the base of the stems (Anon, 1984a). These stems are easily cut and dry matter content is high, so *B. willdenowii* is probably best adapted to silage making (Parneix, 1982b). It has a soluble carbohydrate content similar to italian ryegrass *(Lolium multiflorum* Lam.). The use of *B. willdenowii* on wet soils is not recommended since growth is poor and poaching occurs easily (Betin and Mansat, 1979; Alexandre, 1983). Forage bromes grow well on sands, chalk and other limestones, and heavier soils provided that they are well drained. Soil compaction, due to machinery wheeling in wet weather, has a severely damaging effect. Brome grasses require large amounts of nitrogen fertiliser to exploit their full yield potential. On light soils, phosphate and potash applications are often required at sowing and cutting periods, at rates of 75 kg ha^{-1} per application (Parneix, 1982b).

1.3.4 Cultural techniques

Seeds need to be drilled at a depth of 2 cm into a seedbed prepared as for cereals. Certain varieties, especially Grasslands Matua, have long-awned seeds, which are difficult to sow with most types of drill. Broadcasting the seed, however, is not satisfactory because of the need to incorporate the seed. Recommended seed rates in pure stands are 56 kg ha^{-1} and 40 kg ha^{-1} for varieties Grasslands Matua and Bellegarde (de-awned) respectively.

Red clover *(Trifolium pratense* L.) and lucerne *(Medicago sativa* L.) which are also normally grown for conservation, can be particularly suitable companions to *B. willdenowii* by complementing its growth habit (Frazer, 1982) (Plate 1.4). Seed rates in legume mixtures are in the order of 4:1 (brome:legume). These mixtures will reduce fertiliser use and weed invasion but palatability may be lower than in pure brome. On heavier soils, perennial ryegrass at 7 kg ha^{-1} is complementary to the growth habit of *B. willdenowii* (Barloy, 1982). Grasslands Matua should be particularly attractive to those farmers with well drained chalky or sandy soils, who require good conservation yields or who can utilise the good growth in the late autumn. In these circumstances, it should be a radical improvement on italian ryegrass in terms of both yield and persistence.

1.3.5 Chemical treatments

B. willdenowii is relatively free from leaf diseases but seed dusting with benomyl (6 g kg^{-1} seed) as a precaution against head smut *(Ustilago bullata* Berks.) is advisable.

On established crops of brome, self-seeding restricts weed invasion. The most prevalent weeds in brome swards tend to be chickweed and annual meadow grass *(Poa annua* L.). In pure swards, these weeds can be controlled pre-emergence by neburon at 1.8 kg $a.i.ha^{-1}$ (Magenham, 1983). Up to the tillering stage of the crop, annual meadow grass can be controlled by metoxuron at 6 $kg\ a.i.ha^{-1}$ and broad-leaved weeds by an ioxynil/mecoprop mixture. Once tillering has commenced, chlortoluron at 3 $kg\ a.i.ha^{-1}$ is safe for use against most grass weeds. In brome and legume mixtures, MCPB will give some control of broad-leaved weeds.

Brome swards can be terminated using paraquat or glyphosate. *B. willdenowii* seedlings have not yet proved to be a problem in subsequent crops in France, being easily removed if necessary with ch-lortoluron (pre-emergence) or terbutryne in cereals, ethofumesate in beet, or simazine in beans or maize *(Zea mays* L.) (Anon, 1984a). In practice, *B. willdenowii* has had to be controlled in crops in the U.S.A. (Chenault and Wiese, 1977), and in cereals in S. America (Romero *et al.*, 1969; Rodriguez, 1982). *B. willdenowii* appears to be resistant to several herbicides (Hazard, 1967), especially dinoseb (Sánchez *et al.*, 1970), and it cannot be controlled by metoxuron in Britain (Jarvis, 1982). It proved tolerant to bromoxynil/ioxynil and dichlorprop/MCPA/ioxynil at the 4 leaf stage (Zadoks GS 14) at twice the normal dose (Standell and Haggar, 1985). The only herbicide which caused any noticeable effect was methabenzthiazuron, applied pre-emergence, but it had no effect on final dry weight yield.

1.4 ISOPROTURON

1.4.1 History

The discovery and subsequent development of the herbicidal properties of the substituted ureas began after the Second World War. By 1946, Thompson *et al.* had found 82 urea derivatives with some phytotoxic activity. The majority of these possessed a high order of inherent toxicity and consequently were used mainly as total weed killers. The first substituted urea to be introduced commercially was dichloralurea, marketed by the Union Carbide Corporation in 1950. Bucha and Todd (1951) reported on the success of another urea, monuron, in the control of grass weeds, and it was subsequently marketed by Du Pont as 'Telvar'. It was originally used for total control, but also gave useful selective control in orchards. In more recent years, the relatively primitive phenylureas have largely given way to more complex analogues with enhanced selectivity.

1.4.2 Properties

The herbicidal activity of isoproturon, 3-(4-isopropylphenyl)-1,1-dimethylurea (IUPAC) was first reported by Thizy *et al.* in 1972. It has the molecular formula $C_{12}H_{18}N_2O$ and a molecular weight of 206.3 (Figure 1.3). The physical form of isoproturon is colourless crystals with a melting point of 151 - 153°C. Although the ureas generally exhibit low water solubility and limited organic solvent solubility, isoproturon is readily soluble in water (70 mg ℓ^{-1} at 20°C) and most common organic solvents. It is a non-volatile herbicide and is stable to light and to acids. It has a vapour pressure of 3.3×10^{-5} Pa (33 µPa) and a density of 1.16 at 20°C (Anon, 1984b).

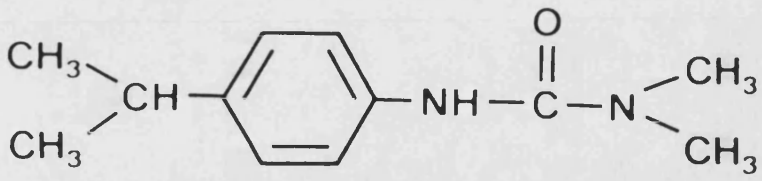


FIGURE 1.3

The chemical structure of isoproturon, 3-(4-isopropyl phenyl)-1,1-dimethyl urea.

Isoproturon has a low oral and dermal toxicity (LD_{50} for rats - acute oral toxicity = 2417 mg kg^{-1} and acute dermal = 2000 mg kg^{-1}). It has no effect on laboratory animals at 400 mg kg^{-1} over a period of 90 days feeding (Fletcher and Kirkwood, 1982).

Isoproturon is manufactured by Rhône-Poulenc, (May & Baker), Hoechst and Ciba-Geigy, and marketed under the trade names 'Tolkan', 'Arelon' and 'Graminon' (Hytane 500L) respectively. These products may vary in performance since their formulations may differ (Okereke *et al.*, 1981). Isoproturon is formulated as a suspension concentrate and a wettable powder, the former type being the most successful (Ingram and Kyndt, 1981).

Isoproturon has a wide compatibility with other herbicides, the most commonly used formulations being isoproturon/bromoxynil/ioxynil ('Doublet' and 'Twin-Tak', May & Baker), isoproturon/ioxynil/mecoprop ('Musketeer', Hoechst, 'Post-Kite', FBC) and isoproturon/trifluralin ('Pre-Kite', FBC).

1.4.3 Uses

Isoproturon has value in the selective control of grass and broad-leaved weed species in winter cereals and spring barley (Elliott *et al.*, 1979). It gives useful control of blackgrass (Black and Hewson, 1978; Moss, 1979; Ayres and Cussans, 1980), and wild oat (Hewson, 1974; Black and Hewson, 1978), both grass weeds being of major importance in cereals. Other annual grass weeds controlled include ryegrasses (*Lolium* spp.) (Hewson, 1974) and the meadow grasses (*Poa* spp.) (Hewson and Read, 1985). Isoproturon is also used for the control of problem broad-leaved weeds such as fat hen (*Chenopodium album* L.), groundsel (*Senecio vulgaris* L.) and poppy (Fryer and Makepeace, 1978). Hewson and Read (1985) reported good control of chickweed, mayweed (*Matricaria* spp.) and black bindweed (*Polygonum convolvulus* L.). Resistance to isoproturon has not been identified (Moss and Cussans, 1985).

Isoproturon is safe for use on all varieties of winter wheat and barley at any stage, even at double the commercial application rates (Hewson, 1974). However, Tottman *et al.* (1975) recommended the use of a maximum of 2 kg a.i.ha^{-1} on winter wheat. They found that several cultivars were intolerant to the related herbicide chlortoluron, but distinctions could not be found between varieties with respect to isoproturon, and they concluded that the variations in response of cultivars are due to the expression of several genetic characters interacting with the environment.

Certain herbicides affect crop species indirectly without producing deleterious effects. Sublethal concentrations of herbicides have been known to stimulate plant growth (Wiedman and

Appleby, 1972; Fedtke, 1973; Ries, 1976). In tolerant crop species, sublethal quantitites of the compound can increase photosynthetic and metabolic capacity (Fedtke, 1973). This was indicated by an increase in the concentration of water soluble protein, amino acids and nitrate (Fedtke, 1974). A simultaneous increase in chloroplast size and an altered pigment composition occurred, plants resembling those grown under low light conditions (Fedtke, 1973; Fedtke *et al.*, 1977). These changes lead to a growth stimulation, a phenomenon that was first recognised by Schulz in 1888, who proposed that all poisons are stimulatory at sub-lethal concentrations. The effect has been reported for diuron (Minshall, 1960), methabenzthiazuron (Fedtke, 1973) and several other herbicides (Wiedman and Appleby, 1972). Changes associated with the addition of isoproturon at sub-toxic levels have not been investigated.

1.4.4 Mode of action

The site of action of the phenylurea herbicides was established soon after their release in 1950 (Bucha and Todd, 1951). Wessels and van der Veen (1956) showed that leaves treated with phenylureas irreversibly lost all ability to assimilate carbon dioxide in the light. They were the first to show that herbicides inhibited photosynthetic electron transfer.

A brief introduction to the relevant aspects of photosynthesis is appropriate here, since it is a pre-requisite for the understanding of the mode of action of the substituted phenylurea herbicides. Many excellent review articles, giving more detail, have appeared, namely those by Trebst and Avron (1977),

Pfister and Arntzen (1979), Vermaas and Govindjee (1981), Kaplan and Arntzen (1982) and Corbett *et al.* (1984).

The path of photosynthetic electron transport generally accepted by most workers is shown in Figure 1.4. It is a modern version of the 'Z' scheme first proposed by Hill and Bendall in 1960. These reactions are located, together with the light harvesting pigments, within the chloroplast thylakoid membranes, and lead to the generation of ATP and the reduced form of the coenzyme NADP i.e. NADPH. ATP and NADPH are subsequently used to reduce CO_2 in the Calvin cycle reactions within the chloroplast stroma.

Light energy is absorbed by two discrete photosystems, the reaction centre chlorophyll of PS I designated as P_{700}, and of PS II as P_{680} (Floyd *et al.*, 1971), (pigments with absorption maxima of 700 and 680 nanometres respectively). Electrons are passed through PS II to the PS I reaction centre by electron carriers of successively lower reducing power, and subsequently to NADP. (A compound with a high negative value on the redox scale (E_o) will be an efficient reducing agent).

The ultimate source of electrons is a water splitting complex at P_{680}, of which oxygen is a by-product. (Protons from water form a gradient in the intra-thylakoid space, which is used to drive the synthesis of ATP, a process termed photosynthetic phosphorylation). The first stable electron acceptor of PS II is Q_A, a quinone which is thought to be a plastoquinone molecule in a special environment. Q_A quenches the fluorescence from chlorophyll P_{680}, and passes electrons on to Q_B, a protein, and possibly also a quinone. Electrons arrive at the plastoquinone pool (PQ) as the

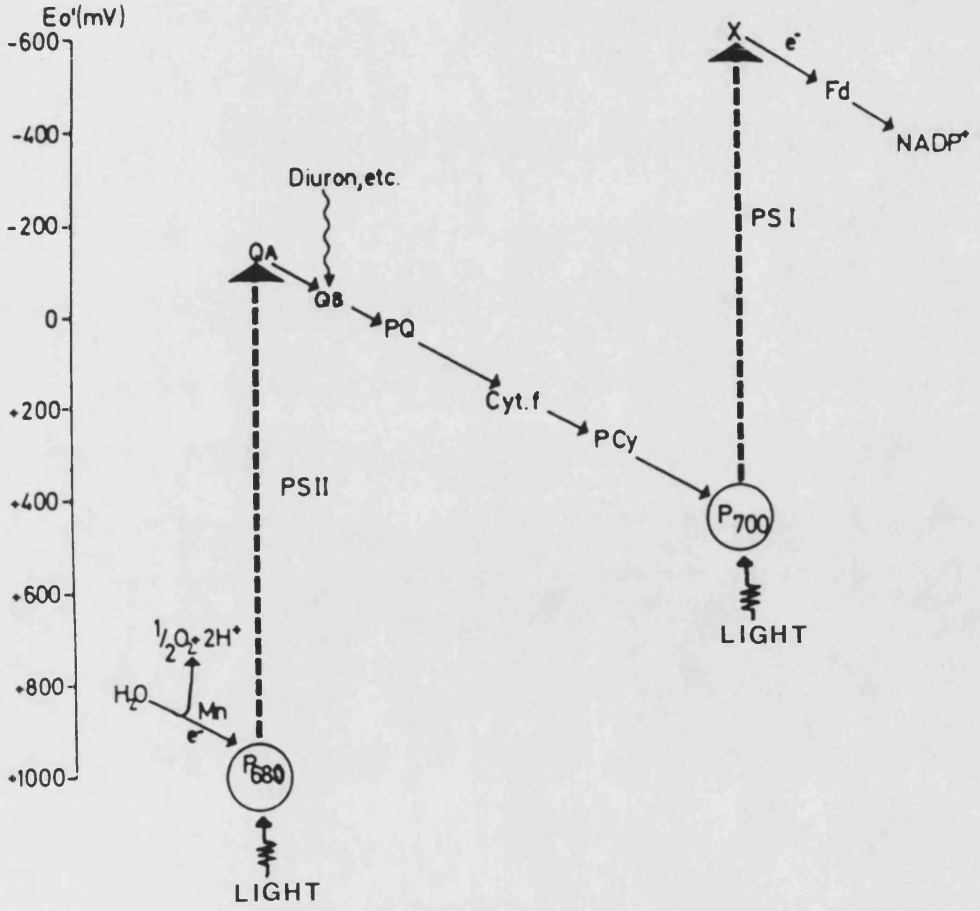


FIGURE 1.4

The photosynthetic electron transport system in chloroplasts. The inhibition site for isoproturon is indicated by a waved arrow. (see text for explanation of abbreviations).

quinol, PQH_2, and from there, pass to P_{700} via an iron-sulphur protein, cytochrome f (Cyt. f) and the copper protein, plastocyanin (PCy). After further energy has been absorbed by PS I, the primary electron acceptor (X) can reduce ferredoxin (Fd), which in turn will reduce $NADP^+$ to NADPH.

Figure 1.5 shows the approximate location of the components of these reactions within the thylakoid membrane (Renger, 1979; Kaplan and Artnzen, 1982).

Isoproturon is a potent inhibitor of the 'Hill reaction', interfering with electron transport on the reducing side of PS II (Duysens, 1964). The site to which most PS II herbicides bind is often referred to as the 'diuron site', since it was initially discovered using diuron (Wessels and van der Veen, 1956). The exact binding site is likely to be between Q_A and Q_B, since Q_A is functional as an electron carrier, but Q_B is not active as an acceptor (Pfister and Arntzen, 1979). Incorrect functioning of the Q_B acceptor could result from displacement of Q_B from its binding site (Corbett *et al.*, 1984), by alteration of the redox potential of Q_B making it more difficult to reduce, or by impairing the $Q_A \rightarrow Q_B$ reduction by allosterically induced shape change (van Rensen, 1982). This binding site occurs at the rate of one per electron transfer chain, and is probably located on a protein of molecular mass about 32 K daltons (Renger, 1976; Moreland, 1980; Trebst *et al.*, 1983). The presence of other membrane components seems also to be required for herbicide-induced inhibition of electron transport (Gressel, 1982). Renger (1976) proposed that the protein shield carrying the binding site was involved in regulating

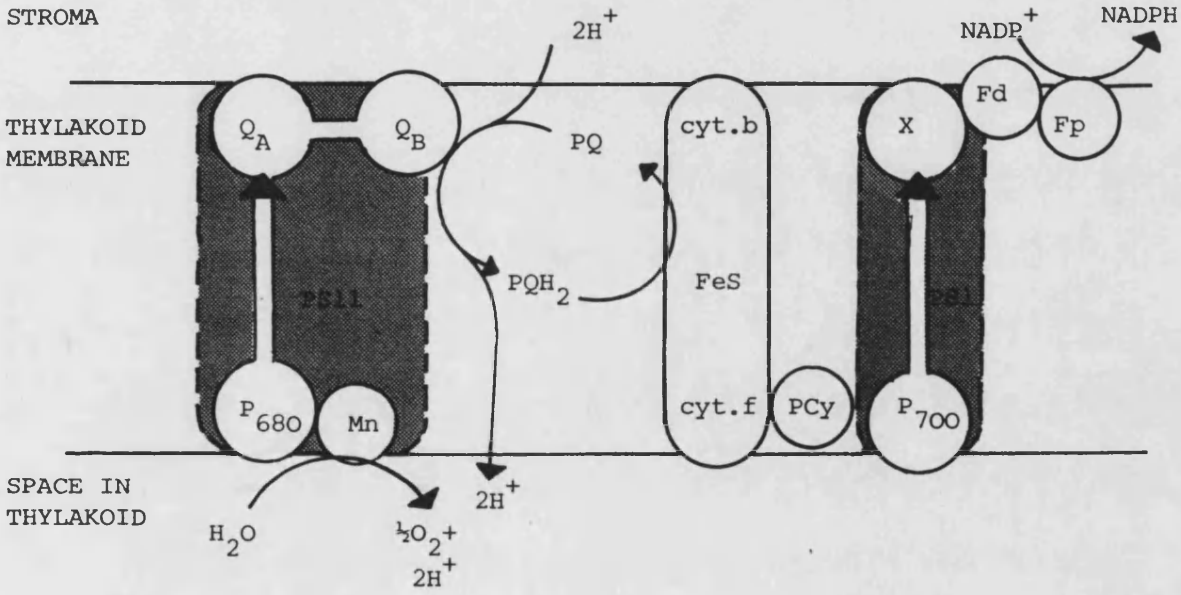


FIGURE 1.5

The arrangement of major components of the photosynthetic electron transport system within the thylakoid membrane. (see text for explanation of abbreviations).

electron flow between Q_A and Q_B. This is likely, since in many plants, the protein is both rapidly synthesised and degraded, and of broadly similar structure (Hoffman-Falk *et al.*, 1982).

A secondary site of inhibition may occur on the oxidising side of PS II for some herbicides (York and Arntzen, 1979), however, the 'diuron site' is the major location for phenylureas (Hatzios *et al.*, 1979). Inhibition of PS I appears to be insignificant.

Besides photosynthesis, other plant metabolic processes may be inhibited directly by phenylureas, though higher concentrations are required for these effects. They have been shown to affect the respiratory system by interfering with ATP synthesis by oxidative phosphorylation (Moreland, 1974), but this is probably incidental to the major mechanism of action.

Inhibition of the Hill reaction prevents ATP and NADPH formation, consequently reducing CO_2 fixation and eventually depleting sucrose levels. However, early experiments have revealed that the lack of assimilates in inhibited plants did not cause the observed damage (Sweetser and Todd, 1961; Ashton *et al.*, 1963; Ashton, 1965). Symptoms induced by the toxic action of phenylureas are generally expressed via the leaves. Chlorosis is followed by more extensive leaf injury, such as wilting, stem collapse and yellowing of leaf veins and margins (Bucha and Todd, 1951; Muzik *et al.*, 1954; Minshall, 1967; Brian, 1976).

Death results from destructive reactions which follow a failure of the mechanism that normally protects the photosynthetic apparatus against excessive illumination (Stanger and Appleby, 1972; Ridley, 1977). In the normal chloroplast, excitation energy

absorbed by the chlorophyll pigments leads to the initial excitation to the short-lived singlet state, with a lifetime of 10^{-8} sec. This state is quenched by rapid energy transfer to the reaction centres P_{680} and P_{700}. If unquenched, because electron transport is inhibited, intersystem crossing may lead to the generation of the longer-lived triplet state chlorophyll, with a lifetime of 10^{-3} sec (Pallett and Dodge, 1980). If the triplet state is unquenched by carotenoid pigments, deleterious reactions may occur in two ways (Figure 1.6):- in type I reactions, triplet chlorophyll directly induces proton abstraction from unsaturated fatty acids to yield lipid free radicals. In more important type II reactions, triplet chlorophyll interacts with triplet oxygen to generate singlet oxygen, which itself induces lipid peroxidation formation. Lipid peroxidation, to which membranes are particularly susceptible, leads to cellular destruction and death (Dodge, 1982; 1983a). Therefore, excitation energy, absorbed and prevented from driving electron flow, overloads the natural carotenoid protective system before proceeding to break down chlorophyll (Pallett and Dodge, 1980). These secondary effects of photosynthetic inhibition are responsible for the visible damage to plant tissue (Dodge, 1983b).

1.4.5 Metabolism

Upon entering a plant cell, a herbicide (a xenobiotic) is likely to be metabolised or inactivated by natural defence systems (Wain and Smith, 1976). Potential fates include binding to cell wall constituents, or within the symplast, to membrane proteins, enzymes or other macromolecules (Naylor, 1976).

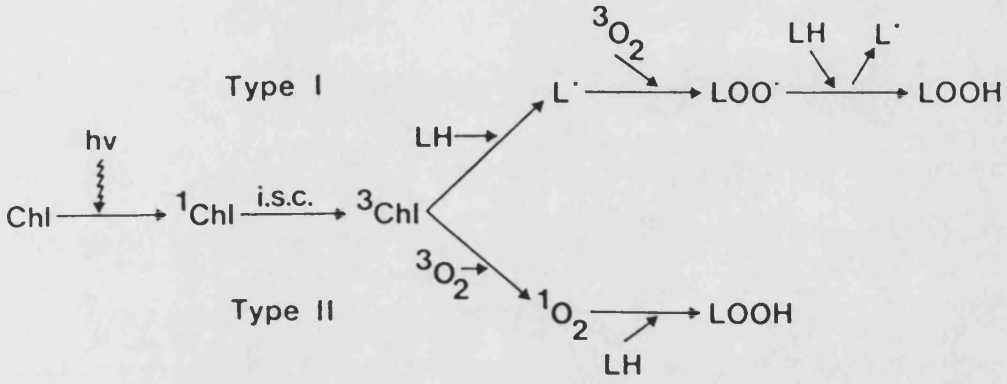


FIGURE 1.6

Representation of damage to unsaturated fatty acids (LH) as a result of electron transport inhibition.
^{1}Chl, singlet chlorophyll; i.s.c., intersystem crossing; ^{3}Chl, triplet chlorophyll; $^3O_2$, triplet or ground state oxygen; $^1O_2$, singlet oxygen; L, lipid free radical; LOO, peroxidised lipid radical; LOOH, lipid peroxide. (from Dodge, 1983).

A herbicide could be partially or completely degraded by enzymes.

Little is known specifically about the breakdown of isoproturon in plants. A number of degradative reactions have been demonstrated for phenylureas, namely N-demethylation, ring hydroxylation and aniline formation (Fletcher and Kirkwood, 1982). N-dealkylation is the major pathway of urea breakdown in plants and soil (Geissbühler *et al.*, 1975). N-demethylation, the most common alkylation reaction in biological systems, is often followed by conjugation of the intermediates as O-glucosides (Ryan and Owen, 1983) (Figure 1.7). Another independent mechanism, ring hydroxylation,has been reported for some ureas e.g. chlortoluron, and appears to be predominant over N-demethylation (Gross *et al.*, 1979). Ryan *et al.* (1981) showed that ring-methyl oxidation reactions gave wheat and barley plants enhanced tolerance to chlortoluron.

Although the selectivity of certain substituted phenylureas has not been elucidated fully (Geissbühler *et al.*, 1975), a differential rate of metabolism, primarily through N-demethylation, has often been proposed as a basis for selectivity in certain plants. Reduced rates of metoxuron degradation in certain varieties of wheat partially accounted for selectivity (Müller and Sanad, 1975; van Leewen and van Oorschot, 1976). However, there was apparently no varietal difference in the rate of isoproturon degradation (Müller *et al.*, 1977).

Fournier *et al.* (1975) showed that isoproturon was normally degraded in soil by microbial rather than by metabolic processes. Isoproturon follows the pattern of other phenylureas with respect to breakdown in soil, the major pathway being dealkylation

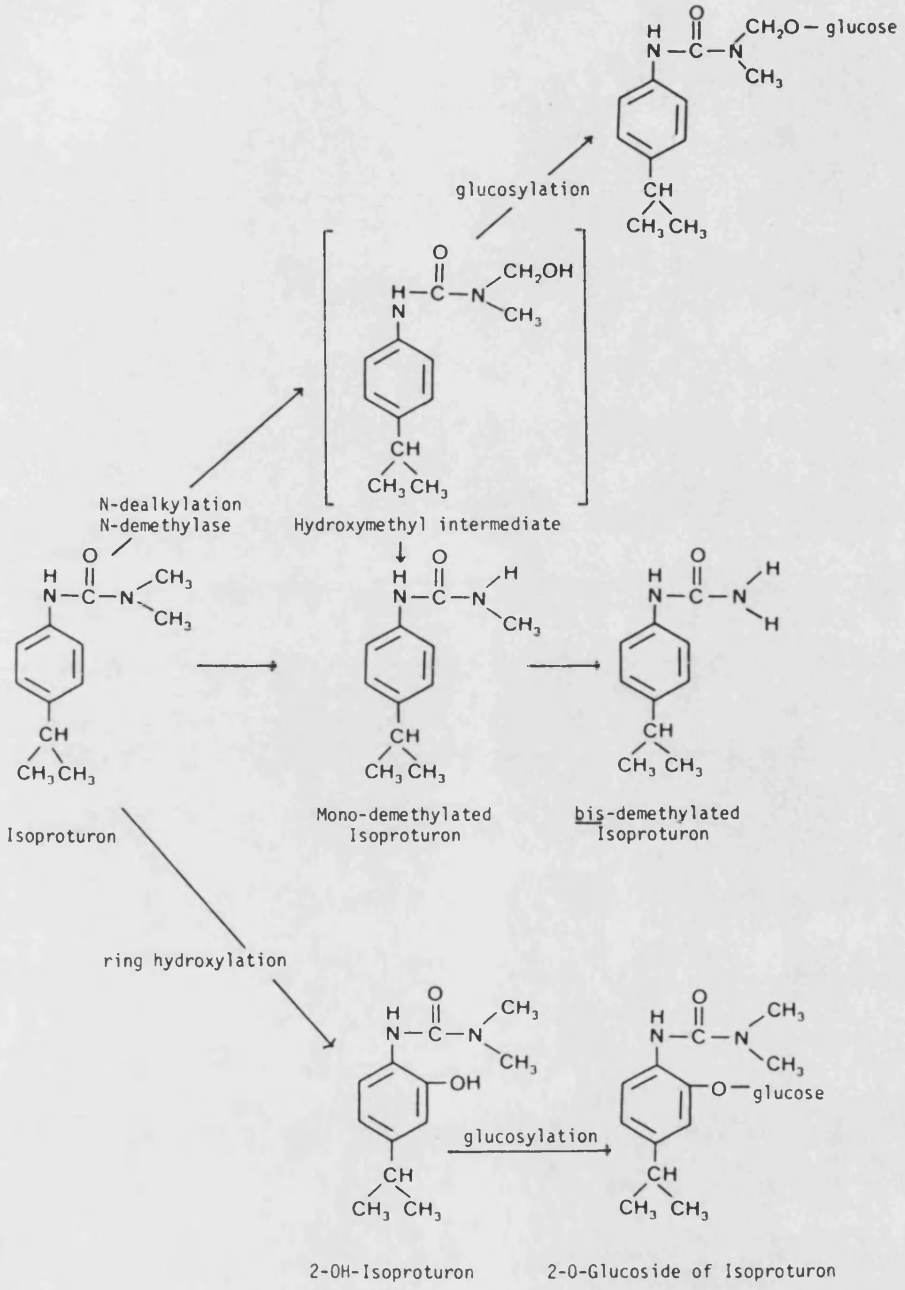


FIGURE 1.7

The metabolism of isoproturon in higher plants. (adapted from Hatzios and Penner, 1985).

followed by formation of the corresponding aniline (Geissbühler *et al.*, 1975; Mudd *et al.*, 1983). Gross *et al.* (1979) showed that ring-methyl oxidation did occur in soil, yet N-dealkylation predominated.

1.4.6 Persistence

Persistence depended upon the formulation and method of application, its transport in soil, and degradation by micro-organisms (Furmidge and Osgerby, 1967). The persistence of isoproturon was variable in field tests, for example, Moss (1979) recorded that 2 - 8% of an autumn application remained the following spring, and other reports recorded 40 - 80% (Anon, 1979). Under average soil conditions of temperature and moisture, the persistence of herbicidal activity has been shown to last for some 6 - 10 weeks (Anon, 1977). Soil type will influence breakdown, clay soils supporting greater persistence than fine loam soils (half-lives 38 and 23 days respectively) (Kulshrestha, 1983). The moisture content of soil directly influenced the persistence of isoproturon. The dissipation of the herbicide in air-dry soil was half the rate in soil containing moisture at field capacity (half-lives 43 and 26 days respectively). In flooded soil, the half-life was only 11 days (Kulshrestha, 1983). Rainfall was important in the movement of isoproturon within the soil profile (Luscombe, 1981). Isoproturon tended to be mobile after heavy rain, falling to a depth of 16.2 cm after 10 cm of rain (Luscombe, 1983).

Temperature did not appear to be an overriding factor affecting the persistence of isoproturon activity (Luscombe, 1983).

1.4.7 Environmental factors

Several environmental factors have a profound effect, both directly and indirectly, upon the toxicity of isoproturon. Light is thought to be a prominent factor influencing the degree of plant damage by a herbicide, such that as light intensity increases, plant damage increases. Blair *et al.* (1983) found that *B. sterilis* was less damaged under 70% shading than at higher light levels when sprayed with metoxuron. Seasonal variations in light intensity, and canopy structure with respect to shading, may influence the phytotoxicity of isoproturon to *B. sterilis*. Plants develop thicker and waxier cuticles with increases in light intensity (Baker, 1980), having an indirect effect upon retention. Rates of uptake and translocation may be affected by light intensity (Leonard *et al.*, 1968).

The degree of weed control is also influenced by the temperature regime after herbicide spraying. In general, high temperatures before and after spraying appear likely to increase weed susceptibility and mortality, but super-optimal temperature may reduce foliar entry by causing wilting, stomatal closure and rapid evaporation of spray droplets. The activity of isoproturon against *B. sterilis* was substantially decreased as the post-~~emergence~~ spraying temperature regimes were increased from 10°C/6°C to 26°C/16°C (D/N) (Okereke *et al.*, 1981b). Ayres and Richardson (1981) also reported that *B. sterilis* sustained less damage at high temperatures. Ureas are generally more active on *B. sterilis* in a

cooler post-spraying environment such as 10°C/6°C (Blair *et al.*, 1983). The mechanism by which temperature affects isoproturon activity is unclear. Gerber *et al.* (1983) suggested that the greatest amount of a photosynthetic inhibitor herbicide is taken up at the optimum temperature for growth of the particular plant species. Uptake is directly related to the transpiration rate, which will be reduced by low temperatures (Muzik *et al.*, 1954; Penner, 1971). There is a specific temperature at which optimum uptake and translocation of the herbicide occurs for each species, for example, wheat plants achieve their optimum at 20°C and wild oats at 15°C (McIntosh *et al.*, 1981). Okereke *et al.* (1981) suggested that the effect of temperature may be related to the balance between rate of accumulation of the active ingredient of the herbicide and its degradation within the foliage.

The relative humidity will affect the plants' water stress, stomatal opening and cuticular permeability (Currier and Dybing, 1959). Toxicity of a herbicide is generally expected to decrease with growing humidity, since there is a decreased flow of water through the plant in high humidities (Kirkwood, 1977). Blair *et al.* (1983) found however, that there was more damage to *B. sterilis* plants from high relative humidity (95%/98%) than from lower values (75%/86% RH) with metoxuron. It appears that different species of plant have an optimum uptake of isoproturon at different humidities. For wheat plants, optimum uptake occurred at 30% RH, while in wild oats, it occurred at 90% RH (McIntosh *et al.*, 1981). There may be mechanisms other than uptake and translocation that are affected by humidity. The persistence of liquid on leaves is determined by RH, and penetration appears to cease with droplet desiccation (Holly,

1956). Plants develop thicker and waxier cuticles in low rather than high humidities (Verity *et al.*, 1981).

Rainfall or irrigation has been considered of importance for most pre-emergence herbicides to be effective (Stickler *et al.*, 1969). Rain moves herbicides down the soil profile, the volume of water determining the soil moisture content. The soil moisture content has a marked effect upon the phytotoxicity of several urea herbicides (Stickler *et al.*, 1969). Isoproturon activity declines under dry conditions (Ingram and Kyndt, 1981), due to the prevention of migration of the product in soil water. Generally, the toxicity of a herbicide increases with increasing soil moisture content, being the case for diuron (Upchurch, 1957), simazine (Grover, 1966), but not picloram (Grover, 1970). Blair *et al.* (1983) observed more damage to *B. sterilis* from metoxuron at a high soil moisture content. The distribution of soil water is clearly important, since the volume of water required to bring the soil to field capacity, when applied to the soil surface, resulted in good control of blackgrass treated with isoproturon; however, the same amount applied below 4 cm in the pot gave poor control (Blair, 1985). Damage to blackgrass increased with an increase in soil moisture of 50 - 150% F.C. A soil moisture content of 40% F.C. produced plants with more waxy cuticles than those grown at 100% F.C., thus penetration rates were reduced in the former (Baker and Procopiou, 1980). In post-emergence treatments, rain during or closely following spraying will wash intercepted spray from the leaves and reduce its effectiveness in most cases. Traces of rain or dew a few hours after spraying can increase penetration by rewetting herbicidal deposits. Soil moisture will influence not only herbicide

availability, but also water and nutrient uptake and subsequent movement (Wills and Basler, 1971), thus plant growth.

1.5 OBJECTIVES

The programme of research reported here began in 1982, with the intention of determining the relationship between isoproturon and *B. sterilis* in more detail. From pot and field trials, isoproturon has proved to be one of the few herbicides that is effective against *B. sterilis*. It is already employed in many cereal crops, since it controls major weed problems such as blackgrass and wild oat. There are evidently many factors controlling the response of *B. sterilis* to isoproturon, since there is great variability in the results obtained. Experiments were designed to determine the margin of selectivity of isoproturon between *B.sterilis* and the relatively tolerant barley plant.

It is envisaged that *B. willdenowii* may become a problem weed in cereal crops, just as the related *B. sterilis* has done. Its spread from fields where it is grown as a crop, into neighbouring cereal crops, seems inevitable unless preventative measures are taken. For this reason, and for comparison with *B. sterilis*, which has only relatively recently become a problem weed, a study of its response to isoproturon was undertaken.

Herbicidal selectivity can be defined as the ability of a compound to either kill or to inhibit the growth of weeds, while leaving the crop relatively unharmed. Selectivity may result from gross morphological differences connected with the availability of the herbicide to the root or foliar surface, to differential absorption, translocation and inactivation in the tissues of various

plant species, and finally to differences in susceptibility at the chloroplast level (van Oorschot, 1979).

This research investigation can be divided into a number of experimental sections. In each one, a factor that could account for the selectivity of isoproturon between *B. sterilis*, *B. willdenowii* and barley has been examined. Experiments were designed to evaluate the contribution of:-

1. Morphological and physiological differences between species prior to herbicide application.
2. Herbicide application methods, and timing of treatments in relation to the stage of growth of plants.
3. Retention of dyes and leaf surface characteristics to assess the susceptibility to foliage-applied isoproturon.
4. Differential uptake from soil and nutrient culture, identifying the specific site and rate of isoproturon uptake.
5. Herbicide translocation rates and the direction of movement within the three species.
6. Direct effects of isoproturon toxicity to the chloroplast, and rates of recovery from photosynthetic inhibition.
7. Metabolism of isoproturon *in vivo*.

2. OPTIMIZATION OF EXPERIMENTAL CONDITIONS

2.1 INTRODUCTION

Before attempting to make comparisons between plant species, it is important to understand their physiological differences. Without this knowledge, it cannot be ascertained whether variations are due to differential response to herbicides. Morphological characteristics vary between species, but there is a common pattern of growth within the *Gramineae*. Initial seed size may influence the rate of radicle protrusion, soil penetration and subsequent growth. Certain species have a capacity for very rapid growth, though this may be limited to short periods of their life cycle.

Weed seeds often show high % germination, a characteristic which has enabled them to survive and compete with crops. However, seed dormancy, which may be innate, enforced or induced by a variety of environmental stimuli, is common with weeds, and this may alter the pattern and rate of germination. Freshly-shed and stored seeds may also respond differently when exposed to conditions favouring germination. Once germinated, 'soil factors' including the organic matter percentage and the moisture capacity of soil may influence plant growth and development. Even though the seeds germinate, they may fail to reach the surface because emergence can only occur when seed depth is exceeded by the limit of coleoptile extension. For establishment of grass seedlings, there is an optimum seed depth which depends on the position of the growing point, which is governed by the position of the seed in the soil.

In this section, studies were made to investigate:-

i) the germination response of both freshly-shed and stored seeds to various light conditions;

ii) the rate and % emergence of *B. sterilis* seedlings from various soil types;

iii) the % emergence and subsequent plant growth from a range of seed depths in soil.

2.2 MATERIALS AND METHODS

2.2.1 Plant production

2.2.1.1 Seed supply

Seeds of *Bromus sterilis* L. were supplied by the former AFRC Weed Research Organisation, Yarnton, Oxon. They were collected from infested farmland in the Oxford region and stored in hessian sacks at room temperature until required. Freshly-shed seeds of *B. sterilis* were collected from arable farmland in the Cotswold area in August, 1985. Seeds of *Bromus willdenowii* Kunth. were obtained from the NIAB seed handling unit, Cambridge. Seeds of *Hordeum vulgare* L. cv. Maris Otter were obtained from seed merchants. (Maris Otter, a valuable winter malting barley, was chosen here since it shows above average susceptibility to many cereal diseases and moderate susceptibility to the related urea, chlortoluron (Ryan and Owen, 1983)).

2.2.1.2 Plant material

In the majority of experiments, plants were grown in plastic pots of 9 cm diam. and height. 300 g of air-dried soil was packed into pots using gentle pressure. Soil moisture content was standardised throughout at 75% pot capacity by the addition of water of a constant volume every second day to pre-weighed soil. 100% pot capacity is the quantity of water retained by soil receiving a continuous supply of water from a shallow dish (Baker, 1980). Pots were randomly circulated at each watering so as to reduce the variations in fresh weights sometimes seen in controlled environment experiments. Plant material used was chosen for uniformity in size and physiological age to minimise variation between plants.

2.2.1.3 Growth conditions

Throughout this work, all plants were maintained in a 'Conviron S10h' growth cabinet containing both tungsten and high output white fluorescent bulbs. Lighting was supplied for a 12 h period at 150 $\mu Em^{-2}s^{-1}$. Temperature was kept constant at 10°C/8°C (D/N) and relative humidity at 70%/75%.

2.2.1.4 Statistical analysis

Where relevant, statistical analysis has been carried out on the data, with a minimum of three replicates (more usually five) making up a mean value. The standard error of the mean is presented in parentheses beside each figure in tables and as a bar on line diagrams ($SE = \sigma \frac{n-1}{\sqrt{n}}$). In some cases, the standard deviation of the mean is presented, but standard error is preferred since it takes into account the number of observations ($SD = \sqrt{d^2} \div n-1$). Two-way analysis of variance, which separates the total variation present into independent components that may be attributed to one source or another, was used to confirm significance (P = 0.001 denotes 99.9% significance. Significance tables are presented in Appendix 11.2).

2.2.2 Germination studies

% germination of seeds of *B. sterilis*, *B. willdenowii* and barley was recorded after ten days incubation in light or dark conditions at 23°C. 20 seeds were sown in each 9 cm diam. Petri-dish containing one 'Whatman grade 1' filter paper. Each dish was drenched with 5 cm^3 of distilled water using a 'Gilson' pipette. Every two days, 2 cm^3 of distilled water was added to each dish to maintain seed moisture.

Germination conditions consisted of a) an incubator supplying continuous light of 250 $\mu Em^{-2}s^{-1}$, b) a 16 h photoperiod of 25 $\mu Em^{-2}s^{-1}$, or c) total darkness. All incubators were maintained at 23°C.

After ten days, % germination was recorded for each species, and the results are expressed as the mean of 20 replicates. Germination was considered to be complete when the plumule was 2 mm long. Freshly-shed seed germination was recorded after 30 days incubation in the same conditions, since after 10 days, germination had not commenced.

2.2.3 Soil type

Seeds of *B. sterilis* were sown at 2 cm depth in pots containing five different soil types available locally, namely John Innes No. 2, Levington Universal, sand, and Bathampton and Mendip field soils (see Appendix 11.1). Ten seeds were sown, horizontally with respect to the soil surface and evenly spaced within each pot.

% emergence was recorded between 7 and 22 days after sowing, at which time the fresh weights of shoots were measured.

In this section, the term soil is used in a general sense to refer to any type of growth medium.

2.2.4 Seed depth

Seeds of the three test species were sown horizontally at depths of 0, 2, 4 and 6 cm in John Innes No. 2 compost. (A preliminary test showed that there was no significant difference between seeds sown horizontally and vertically within the soil, therefore horizontal sowing was standardised). Ten seeds were sown per pot.

% emergence was recorded 28 days after sowing, at which time shoot fresh weights were measured.

Ungerminated seeds were then repotted at 2 cm depth in the same soil to test for viability. At 28 days, % emergence and fresh weights were determined.

2.3 RESULTS

2.3.1 The effect of light on seed germination

% germination of stored seeds of each species was significantly different at P = 0.001. Irrespective of light conditions, mean % germination totalled 96, 83 and 86% for *B. sterilis*, *B. willdenowii* and barley respectively. Similar results were recorded for seeds maintained in a 16 h photoperiod (Table 2.1). The overall effect of light treatment on germination was significant at P = 0.05. The final % germination of *B willdenowii* seeds was not influenced by light conditions, though seeds germinated more rapidly in a 16 h photoperiod. For *B. sterilis* seeds, % germination increased slightly as the period of exposure to light decreased, and germination was most rapid in the dark. Broadly similar results were recorded for barley, although the error was large.

Freshly-shed seeds of *B. sterilis* showed poor germination after 30 days both in light (8%) and dark (37%) conditions, and were also extremely slow to germinate. At 14 days, germination had not commenced, but after 30 days, 65% of *B. sterilis* seeds had germinated in a 16 h photoperiod.

2.3.2 The effect of soil type on seedling emergence and growth

Figure 2.1 shows the rate and final % emergence after 22 days of *B. sterilis* in various soils. Seedling emergence occurred most rapidly from John Innes No. 2 and Levington compost. Though later established, seeds in sandy soil maintained a rapid rate of emergence. Field soil-grown seeds were in comparison slow to emerge,

TABLE 2.1

The percentage germination of stored seeds of *B. sterilis*, *B. willdenowii* and barley after 10 days incubation at 23°C in a) continuous light, b) a 16 h photoperiod or c) continuous darkness.

species	a)	b)	c)
B. sterilis	92.3(1.5)	95.7(1.2)	99.0(0.5)
B. willdenowii	82.7(4.4)	82.7(3.6)	83.0(2.8)
barley	81.3(4.6)	87.7(3.3)	88.3(4.0)
Freshly-shed seeds after 30 days in the same conditions:			
B. sterilis	7.5(2.6)	65.0(8.5)	36.5(5.5)

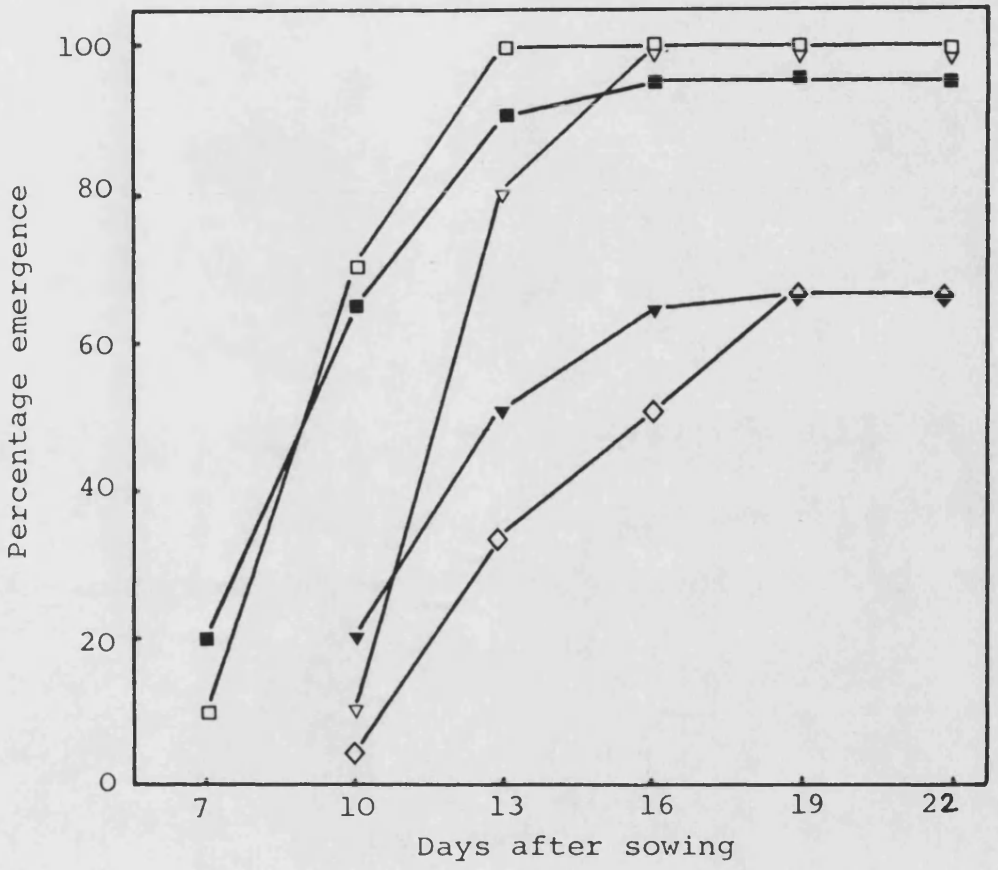


FIGURE 2.1

Rate and % emergence of *B. sterilis* seedlings from different soils.

Levington universal compost (□), John Innes no. 2 (■), sand (▽), Bathampton field soil (◇), and Mendip field soil (▼).

and final % emergence reached only 66%. The formulated composts supported 95 - 100% emergence after 19 days.

Shoot fresh weights of the resulting plants after 22 days are presented in Table 2.2. There was a direct correlation between rates of emergence and final fresh weight. Field soil from the Mendip region produced the smallest seedlings.

2.3.3 The effect of seed depth upon seedling emergence and growth

% emergence of all three species after 28 days was greatest from a depth of 2 cm (Figure 2.2). Below 2 cm, reduction in % emergence directly correlated with seed depth. Preliminary tests with *B. sterilis* indicated that this trend continued, less than 40% emerging from 8 cm and none from below 10 cm (data not shown). The seeds sown at 10 cm had germinated but failed to emerge. Surface-sown seeds exhibited poor germination in all three species, and were significantly different from other treatments at $P = 0.001$. When repotted at 2 cm, % emergence of seeds ranged between 80 - 100%.

For easy comparison, shoot fresh weights of the subsequent seedlings were expressed as a % of the largest mean fresh weight for each species (Table 2.3). Seeds sown at 4 cm depth produced the largest plants on average, though they were not significantly different from those sown at 2 cm. *B. sterilis* seedlings sown at 6 cm were small and weak, whilst surface-sown seeds that did germinate were comparatively larger. *B. willdenowii* and barley fresh weights were unaffected by increasing depth up to 6 cm, but seedlings resulting from surface sowing were poor. Repotted seeds of

TABLE 2.2

Shoot fresh weights of *B. sterilis* plants following 22 days growth in various soils.

soil type	mg plant^{-1}
Levington compost	73.9 (2.0)
John Innes no. 2	61.0 (4.1)
sand	43.3 (2.1)
Bathampton field	41.6 (2.6)
Mendip field	35.3 (1.2)

TABLE 2.3

Shoot fresh weights of *B. sterilis*, *B. willdenowii* and barley plants following 28 days growth in soil at 0, 2, 4, and 6 cm depth, expressed as a % of the greatest weight for each species.

species	seed depth (cm) 0	2	4	6	repotted 0→2
B. sterilis	88.8	100.0	92.5	67.0	78.8
B. willdenowii	46.1	92.1	100.0	98.0	481.8
barley	34.9	82.0	100.0	99.0	208.8

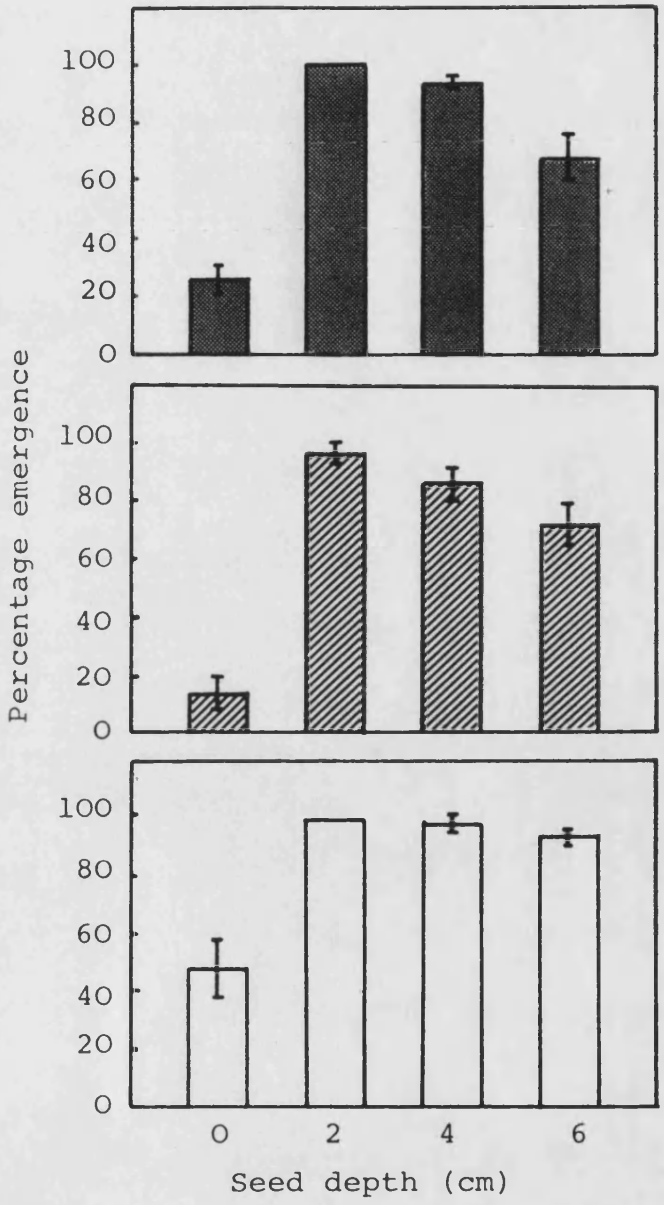


FIGURE 2.2

% emergence of seedlings following 28 days growth in soil at 0, 2, 4, and 6 cm depth. *B. sterilis* , *B. willdenowii* and barley .

B. willdenowii and barley showed an increased fresh weight over the original value at 2 cm after 28 days. Seedlings of *B. sterilis* were larger than those produced from 6 cm depth.

2.4 DISCUSSION

i) *B. sterilis* seeds are very fertile, despite their specific name which implies that they are sterile. These seeds have little or no innate dormancy, as indicated by the rapid germination of stored seeds under all conditions. Froud-Williams *et al.* (1984) recorded slightly lower figures of 81 and 90% germination of stored seeds in red light and darkness respectively. Both sets of results indicate a minimal effect of light on final % germination, possibly due to loss of light inhibition during storage at room temperature (Hilton, 1984). Pollard (1982) noticed that seeds stored in the light prior to water addition germinated more slowly than when stored in darkness. He also reported that stored seeds germinated faster than those collected from hedgerows. These results suggested that partial dormancy had been enforced by light. In the present experiment, the continuous high intensity light of the growth cabinet may be the cause of the apparent rudimentary dormancy in freshly-shed seeds, which required 30 days to germinate. Froud-Williams (1981) noted a similarly slow response in freshly collected seeds of *B. sterilis* shed in June, and Hulbert (1955) recorded this effect in other *Bromus* spp. However, Grime and Jarvis (1975) observed that germination rates did not differ between light, dark or shade-sown seeds. Discrepancies between these results could be related to the differences in light intensity used during experimentation.

In the present study, the rate and final % germination of fresh *B. sterilis* seeds was greater in the dark than in continuous light. This result may reflect differences in the ratio of P_{fr} : P_r in the seed. Phytochrome cannot be phototransformed when in a dehydrated state (Kendrick, 1976). Upon imbibition in red light, P_r

is converted to P_{fr}, the active form of phytochrome, which is directly inhibitory to germination of *B. sterilis* seeds (Hilton, 1982). The weed seeds have been found to contain very little P_{fr} naturally, and because they do not produce P_{fr} in darkness (Hilton, 1984), seeds kept in the dark can germinate rapidly. Possible reasons why % germination was highest in seeds in a 16 h photoperiod include the suggestion that discontinuous, low intensity light may not allow the build up of large amounts of P_{fr}.

B.willdenowii and barley seeds were almost totally insensitive to the light conditions imposed. Selection processes have favoured the individuals in a population that have the ability to germinate quickly, thus light sensitivity may be a disadvantageous trait, and would not be expected to exist in cultivated seed.

ii) Germination of *B. sterilis* seeds occurs naturally in a wide range of soil types. Froud-Williams (1982) found no obvious relationship between brome occurrence and soil type, though heavy infestations occurred in general on calcareous clay soils overlying chalk or limestone. This correlation may be more a reflection of the farming system, since on shallow, stony soils, direct drilling may be preferred to ploughing before seedbed preparation. The differences in plant size, following seed germination in a range of soil types presented here, may result from variations in the soil moisture and organic matter contents. Levington compost, a formulated peaty substrate, has a high organic matter content and a high capacity for moisture retention. Organic matter will retain heat produced as a result of biologial activity, thus maintaining a higher temperature for germination. Soil moisture content can

influence the morphological characteristics of plants, for example, a soil at 100% field capacity produces larger plants with less waxy leaves than a soil at 50% F.C. Sandy soils are free draining, therefore seed imbibition may take longer, as indicated by the delay in emergence of *B. sterilis* seeds from sand (Figure 2.1). The similar response of seeds in the two field soils, their low final % seedling emergence and lower fresh weights, may perhaps be attributed to a deficiency in soil moisture and mineral nutrients. The formulated composts contain high levels of all the essential elements for plant growth, being designed to produce vigorously growing plants.

For experimental purposes, John Innes No. 2 compost was chosen as the standard substrate, since it supported a high % emergence and grew vigorous plants. With an organic matter content of 9.5%, and a field capacity of 27% moisture content, it more closely simulated the type of soil where *B. sterilis* occurs than did the peat-based compost.

iii) Seed depth is often an important factor determining seed germination, emergence and subsequent growth of plants. Radicle protrusion and soil penetration are extremely rapid in *B. sterilis*, which explains its competitive ability in winter cereal crops. Establishment was improved by seed burial to a depth of 1-2 cm (Froud-Williams *et al.*, 1980). The results of the present study indicated that surface-sown seeds of all three species showed poor germination. A less extreme effect was noted by Froud-Williams *et al.* (1984), where 92% of surface-sown seeds of *B. sterilis* germinated, as compared with 95% at 2.5 cm depth. In seeds of

B. sterilis, light inhibition may partially account for the observed reduction in % germination, however, since the other species were also affected, insufficient moisture would seem a more likely cause. The high % germination of all repotted surface-sown seeds at 2 cm supports this theory. Seedlings that did germinate on the surface were much reduced in size, possibly due to inadequate structural support from the soil and lack of a constant moisture supply. The increased size of seedlings of *B. willdenowii* and barley following repotting of surface-sown seeds may perhaps be explained by their longer exposure to moisture, resulting in earlier imbibition.

Okereke *et al.* (1981a) reported a decrease in % germination of *B. sterilis* seeds as burial depth was increased from 5 - 15 cm. A slower rate of emergence also occurred with increased depth, as would be expected. Gray (1981) proposed that any delay in seedling emergence would reduce the competition from *B. sterilis* plants, stressing the delaying effect of soil inversion in farming practices. Emergence of *B. sterilis* has been recorded from depths of up to 13 cm, which can be related to the estimated limit of coleoptile extension (Froud-Williams, 1981). However, Budd (1980) obtained a mean length of 4.34 cm for coleoptiles from seeds sown at 15 cm below the soil surface, and suggested that burial to a depth of 10 cm might be sufficient to prevent seedling emergence. The results of the present study support the conclusions of Budd (1980) in that seedling emergence of *B. sterilis* was reduced from depths greater than the mean coleoptile length of 4.34 cm. Seedlings emerging from 6 cm depth were much less vigorous than those emerging from 2 cm (Table 2.3), indicating that perhaps the seedling leaves had been forced through soil without the protection of the coleoptile.

Increasing seed depth did not have such a marked effect upon *B. willdenowii* and barley seedlings, presumably because seed size was greater and therefore reserves sustained growth for a longer period. The recommended sowing depth for barley seeds is 2 - 3 cm, and for *B. willdenowii*, 2 cm (Parneix, 1982a). Therefore, a sowing depth of 2 cm, which produced equally vigorous plants of all three species was standardised in all subsequent experiments. This standardisation of experimental conditions justifies comparisons being made between *B. sterilis*, *B. willdenowii* and barley.

3. HERBICIDE APPLICATION

3.1 INTRODUCTION

The effectiveness of weed control and degree of crop safety obtained depend to some extent upon the method of herbicide application. Chemicals can be soil- or foliage-applied, or incorporated directly into the soil. A particular species, be it weed or crop, may respond more to either soil or foliage application of a given compound, and herbicides may be formulated accordingly. Many factors contribute to the performance of a herbicide in the field. Isoproturon is generally regarded as acting mainly via the soil (Richardson *et al.*, 1977), although Blair (1978) suggested that foliage uptake may be of some importance under certain environmental conditions.

The wide variations seen in plant response when a herbicide is applied to the soil at different times must reflect differences in the amounts available to the plant (Walker, 1971). Under average soil conditions of temperature and moisture, isoproturon activity has been shown to persist some 6 - 10 weeks (Anon, 1977). Yet persistence depends to some extent on formulation and method of application, as well as its movement in soil and degradation by micro-organisms (Furmidge and Osgerby, 1967). The most advantageous placement of a soil-applied herbicide depends on the chemical and physical properties of the herbicide, soil characteristics, climatic factors, and the site of uptake for the plant species. Soil type and structure influence herbicide decomposition, adsorption on to soil colloids, and the degree of leaching (Lambert, 1966), and these are also markedly affected by the environment. Adsorption is a transient fixation of a dissolved or vaporous substance on or in the surface

of a solid or liquid (Hartley, 1976). If adsorption is reversible, it extends the period over which the herbicide becomes available, thus prolonging persistence. Differences in phytotoxicity between species can be related to the development of the seedling roots in relation to the position of the herbicides in the soil (Blair, 1978).

The effectiveness of a foliage-applied herbicide is broadly dependent upon the properties of the spray formulation, environmental conditions at the time of application, and the chemical and physical characteristics of the target surface (Robertson and Kirkwood, 1969). The efficiency of cuticle retention and penetration, absorption and translocation will influence phytotoxicity and may account for selectivity.

Selectivity between tolerant and susceptible plants may arise out of temporal differences in exposure to the active ingredient of a herbicide. Susceptibility will, to some extent, depend upon the morphology and growth characteristics of a plant at treatment, thus the stage of growth of the weed and crop will influence its control and safety respectively (Roberts, 1982). Susceptibility generally decreases as the age of a plant increases (Blackman and Roberts, 1950; Hammerton, 1967), though exceptions have been reported (Hammerton, 1966). Increased tolerance with age has been explained in terms of a 'growth-dilution' hypothesis (Hagimoto and Yoshikawa, 1972). This postulates that there is a decrease in the tissue concentration of herbicide, resulting from root uptake, as the plant increases in size. However, plants of a greater physiological age offer a larger surface area than young seedlings for foliar uptake of herbicides. Nevertheless, retention of spray liquid, relative to

the mass of the plant, generally decreases with age (Blackman *et al.*, 1949), as the development of cuticular material progresses and wettability decreases (Ashworth and Lloyd, 1961). Gross morphology and leaf surface characteristics vary greatly between species, and for any species can vary with age (Verity *et al.*, 1981).

Pre-emergence applications rely, for weed control, on the herbicide reaching germinating seedlings. Dormant and non-germinated seeds are generally not affected, yet certain herbicides can penetrate the seed coat, and an effect on germination is possible (Åberg and Steckó, 1976). Herbicides are most effective if they are applied when the plants are either growing rapidly or have been weakened by a rapid growth which has temporarily depleted their reserves i.e. seedling emergence prior to chlorophyll synthesis. This implies that the early seedling stages are most vulnerable to herbicide action.

Several workers have investigated the performance of pre- and post-emergence applications of isoproturon on *B. sterilis* (Richardson *et al.*, 1977; Ayres and Richardson, 1981; Pollard and Richardson, 1981; Cussans *et al.*, 1982). In general, these suggest that post-emergence applications were more effective.

The experiments carried out in this section were designed to determine:-

i) the persistence of isoproturon in the test soil;

ii) the effect of isoproturon on seeds of the three species;

iii) the relative importance of soil- and foliage-applied isoproturon in causing damage to the three species under study;

iv) the importance of stage of growth of each species at application.

3.2 MATERIALS AND METHODS

3.2.1 Preparation

3.2.1.1 Isoproturon

Isoproturon, formulated as 'Tolkan' (May & Baker), a suspension concentrate containing 50% w/v isoproturon, was supplied by May & Baker Limited. Suspensions were prepared in distilled water immediately before use.

Throughout these experiments, the amount of isoproturon applied to a pot, regardless of the application method, is expressed in kg a.i.ha^{-1}, giving some indication of the relationship between the dose applied and recommended field rates.

3.2.1.2 Plant material

All seeds were pre-germinated in sealed glass trays (26 cm^2) containing filter paper moistened with distilled water. Seeds of *B. sterilis* and barley were found to germinate rapidly when incubated at 23°C in the dark for 4 days (see Section 2), and these conditions were adopted as standard. Seeds of *B willdenowii* were incubated at 23°C in a 16 h photoperiod supplying 25 $\mu Em^{-2}s^{-1}$ for 6 days.

3.2.1.3 Growth substrate

John Innes No. 2 compost was chosen as the substrate for pot trials because emergence from field soil was too variable (see Section 2). Ten pre-germinated seeds were transferred from glass trays into each pot and buried at a constant depth of 2 cm from the soil surface. Soil moisture content was standardised throughout by watering overhead every second day to return soil to 75% pot capacity.

3.2.1.4 Growth conditions

All plants were grown under the conditions described in Section 2.2.1.3.

3.2.2 Persistence of isoproturon

Pots of soil were prepared in the usual way but without seeds. Half of these pots were treated with 5 kg a.i. ha^{-1} isoproturon as a soil drench. The soil was maintained at 75% pot capacity throughout the experiment.

On the day of application, and every 21 days thereafter, the amount of isoproturon remaining in the treated pots was estimated using the following bioassay technique:- On each assay date, eight treated and four untreated pots were selected at random. Dilution series were prepared from the soil in each of four treated pots in the following manner:-

i) the contents of the pot (300 g of treated soil) were thoroughly mixed with 300 g of untreated air-dried soil in a 20 x 25 cm polythene bag. The air-filled bag was vigorously shaken for 30 sec.

ii) 300 g of the mixed soil was removed from the bag and placed in a pot, forming the 0.5 dilution of the series.

iii) a further 300 g of air-dried soil was mixed with the soil remaining in the bag. 300 g of the mixture was again removed and placed in a pot, forming the 0.25 dilution.

iv) repeated addition of air-dried soil yielded the 0.125 and 0.0625 dilutions.

The soil from the four remaining treated pots was emptied into individual polythene bags, thoroughly shaken, and returned to its pot, thus forming a 1.0 dilution. The soil from each of the four untreated pots was similarly treated and formed the control.

In the process of filling these pots, five pre-germinated seeds of *B. sterilis* were sown at 2 cm depth in the soil of all control and dilution series pots. The shoot fresh weights of these five plants in each pot were determined 17 days after sowing.

3.2.3 Application to seeds

The % germination of dry, stored seeds was recorded after direct contact with isoproturon. Twenty seeds were sown per 9 cm diam. Petri-dish containing one 'Whatman grade 1' filter paper. Control dishes were drenched with 5 cm^3 of distilled water. Treatments consisted of either 0.5 mM or 1.0 mM solutions of isoproturon per dish. Five Petri-dishes were prepared for each treatment. The seeds were incubated in either continuous light of 250 $\mu E\ m^{-2}s^{-1}$, a 16 h photoperiod of 25 $\mu E\ m^{-2}s^{-1}$, or continuous darkness. All treatments were maintained at 23°C, and distilled water added every second day to keep the seeds moist.

The % germination was recorded after ten days, germination considered to be complete when the plumule was 2 mm long.

3.2.4 Application to soil

Isoproturon was applied to the soil surface without contamination of the aerial parts of the plant. This was achieved by dissolving the herbicide in 50 cm^3 of distilled water and dispensing the solution as evenly as possible over the surface using a 5 cm^3 'Gilson' pipette.

3.2.5 Application to foliage

A laboratory sprayer consisting of track and moving nozzle was employed (Plate 3.1). Isoproturon was applied as an aqueous suspension to pots from a height of 47 cm through a 'Teejet 80015 LP' nozzle with a swath width of 60 cm. The volume rate of the herbicide was 610 ℓha^{-1} and the pressure was 0.28 MPa. Plants were left to dry for 30 min before returning them to growth cabinets.

The term foliar application throughout this section refers to overall spray application and the term is used to differentiate it from a high volume soil drench.

3.2.6 Timing of treatments

The timing of soil and foliar applications was determined by the growth stage of the plant concerned, and related to field application stages. Isoproturon was applied to all three species at the following stages according to the Zadoks scale (Zadoks *et al.*, 1974) (Figure 3.1):-

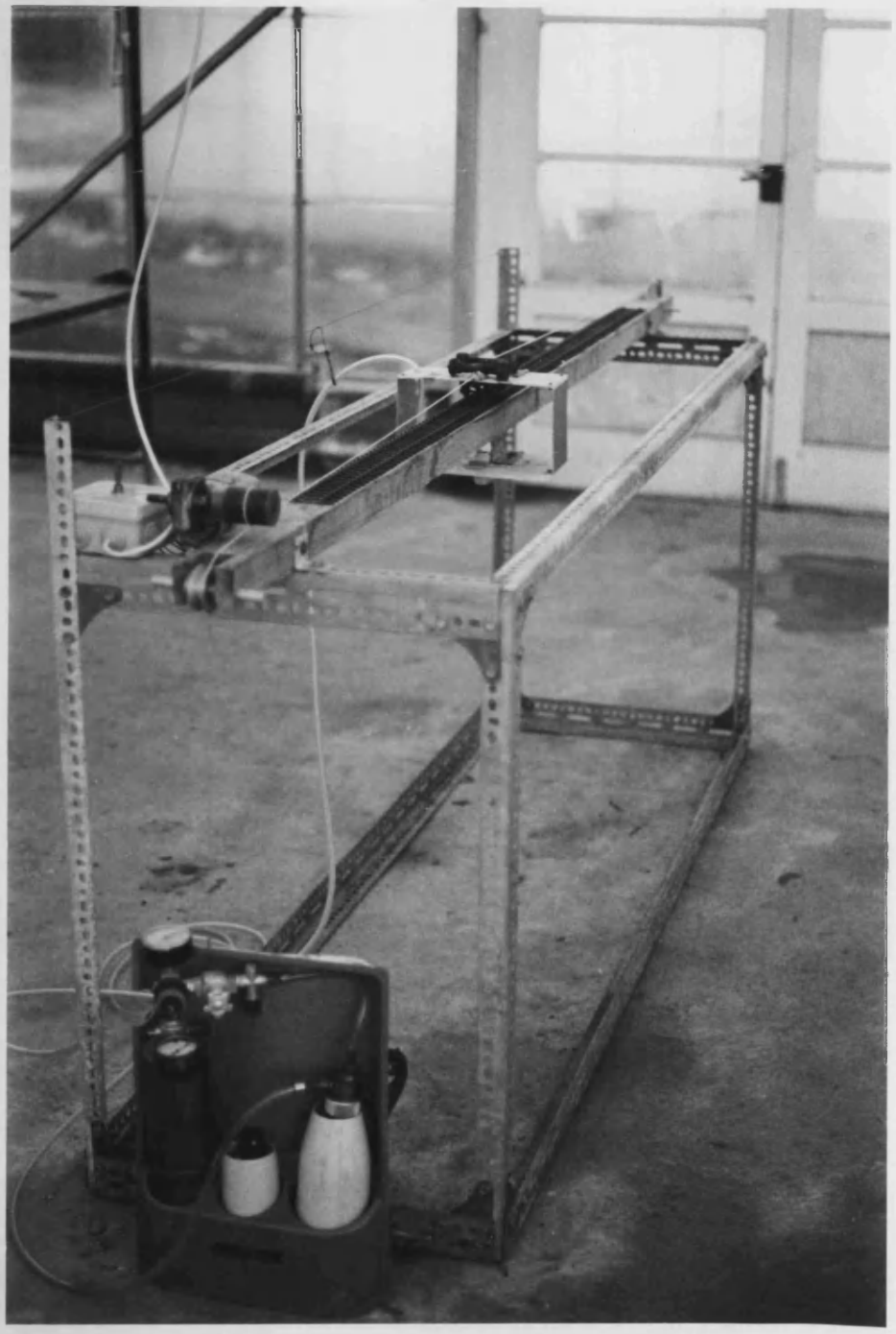
	Zadoks scale
a) Pre-emergence (at sowing)	GS 00
b) Emergence (1 cm above soil surface)	GS 09
c) 1st leaf	GS 11
d) 2nd leaf	GS12-13
e) primary tillering	GS 21

A particular leaf stage was considered to begin when the leaf concerned was fully extended and the following leaf was visible but unextended. The three species reached these stages at different times after sowing, but treatment was irrespective of physiological age.

At each stage, five pots were treated with a soil drench and foliage spray at a range of isoproturon concentrations. Control treatments consisted of distilled water applied to five pots in the same manner as the herbicide.

PLATE 3.1

The hydraulic sprayer used in section 3.2.5.



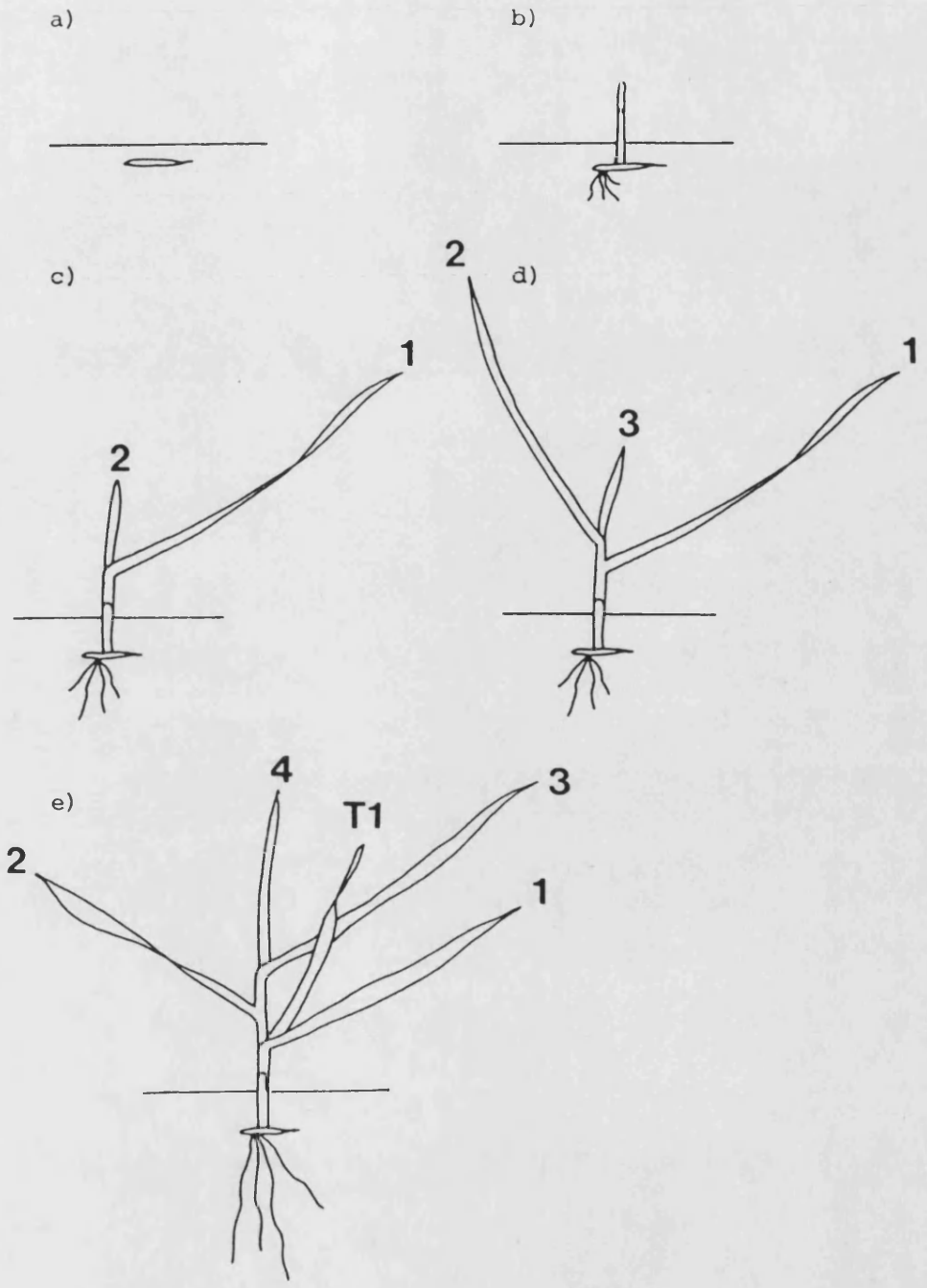


FIGURE 3.1

Growth stages of cereals and grasses. a) 00, b) 09, c) 11, d) 12-13, e) 21. (after Zadoks *et al.*, 1974).

3.2.7 Phytotoxicity assessment

The phytotoxicity of isoproturon was determined by measuring the effect upon fresh weights of plants. Shoot fresh weights were measured immediately following excision at seed level. Harvesting was carried out ten days after treatment. Individual fresh weights of the ten plants in each pot were added, then the sum divided by ten to give a mean value of plant weight per pot. The final value represents the collective mean of five pots.

3.3 RESULTS

3.3.1 The persistence of isoproturon in soil

Figure 3.2 shows the mean shoot fresh weight, expressed as a % of that of the control for each dilution, plotted against the log of the dilution series. The activity of isoproturon remained relatively constant in soil over the 84 day period. Loss of activity was greatest between 0 and 21 days, but the overall loss was negligible, and statistically non-significant. Fresh weights of *B. sterilis* plants decreased as the concentration of isoproturon in the soil increased. Initially, low rates of isoproturon actually stimulated the growth of treated plants. The dose response curve was linear from about 100% at 0.3 kg a.i.ha^{-1} to 30% at 5 kg a.i.ha^{-1}.

3.3.2 The phytotoxicity of isoproturon to seeds

Figure 3.3 shows the % germination of stored seeds of the three species when isoproturon was applied directly, making seed contact. Irrespective of the light condition imposed, the three species differed greatly in their response to isoproturon, and were significant at P = 0.001. Germination of *B. sterilis* seeds was not significantly inhibited by isoproturon in darkness, but in continuous light, germination was reduced to 71% and 28% of the control at 0.5 mM and 1.0 mM respectively. By contrast, seeds of *B. willdenowii* showed poor germination in darkness (19%), and continuous light (18%). Though this figure reached 24% in a 16 h photoperiod, light treatment had no significant effect on this species, being most susceptible to isoproturon.

Barley seeds were the most tolerant of isoproturon under all conditions, yet % germination of untreated seeds was poor. In most

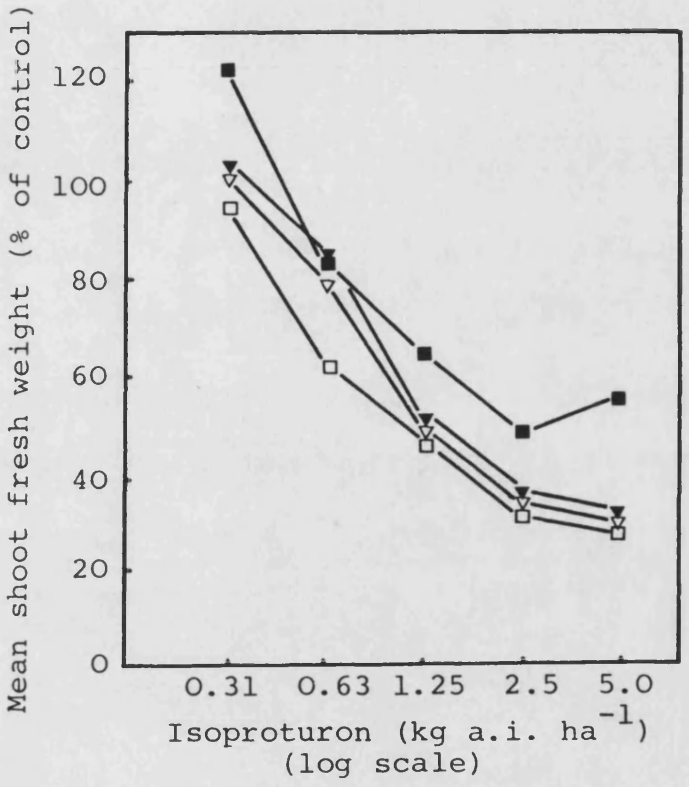


FIGURE 3.2

The persistence of isoproturon in soil at various concentrations, as determined by reductions in growth of *B. sterilis* plants. 0 (■), 21 (□), 42 (▼), and 84 (▽) days after treatment.

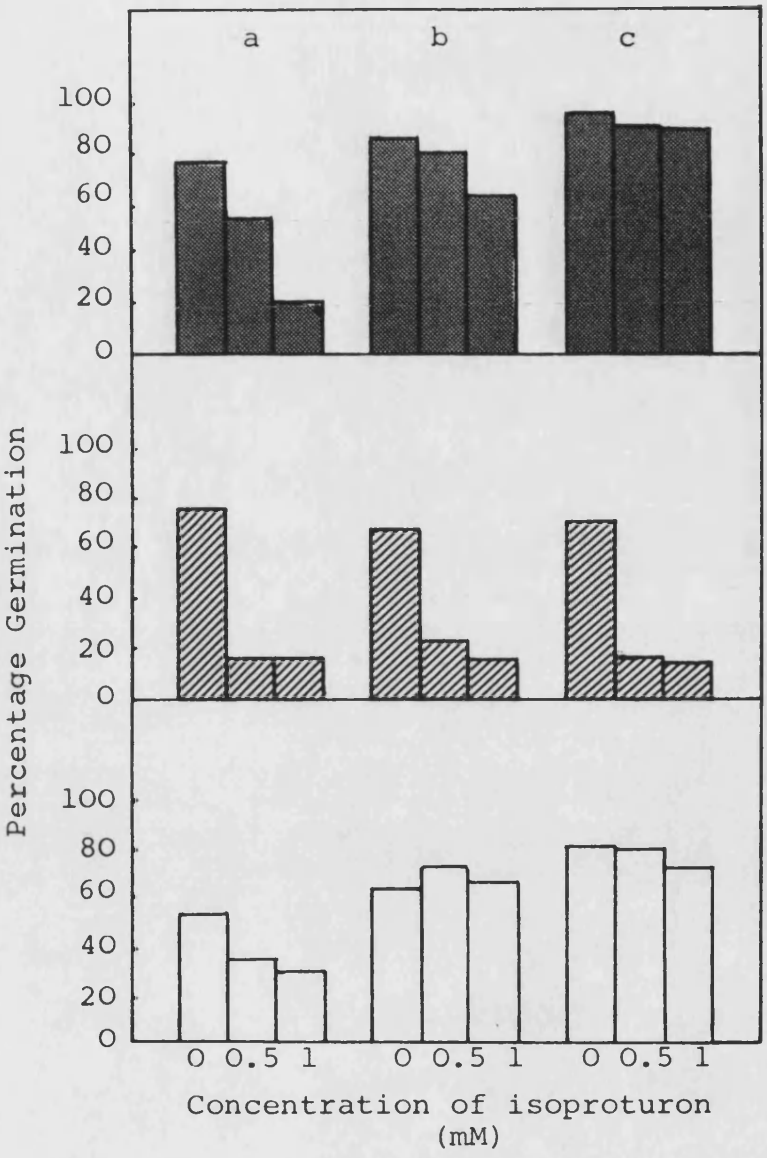


FIGURE 3.3

% germination of seeds after 10 days in either a 0, 0.5 or 1.0 mM concentration of isoproturon, incubated at 23°C in a) continuous light, b) a 16 h photoperiod or c) continuous darkness. *B. sterilis* , *B. willdenowii* and barley .

cases, the stronger concentration of isoproturon, despite being double the strength of the other, had only slightly greater effects upon seed germination percentage.

3.3.3 The effect of stage of growth on the phytotoxicity of isoproturon following root exposure

Figure 3.4 a - e shows the effect of soil drench applications at various stages of growth. *B. sterilis* was the most susceptible of the three species to isoproturon at all rates and stages tested, with the exception of tillering (Figure 3.4e). At the pre-emergence stage, fresh weight reductions were small and insignificant (weights exceeded 72% of the control). At the early post-emergence stages, however, these reductions were larger and distinct from those of *B. willdenowii* and barley at every rate. Applications at the 2nd leaf stage were less phytotoxic than at the 1st leaf stage, and at tillering, the effect of isoproturon was negligible, except at extremely high dose rates. At the recommended field rate of 2.5 kg a.i.ha^{-1}, fresh weights of treated plants were 34% and 26% of those of untreated plants at emergence and 1st leaf stages respectively. At low rates of 0.25 and 0.75 kg a.i.ha^{-1}, isoproturon did not reduce shoot growth below 58%. Conversely, there was so little difference between fresh weight reductions at 2.5 and 7.5 kg a.i.ha^{-1}, that rates in excess of the former were not applied in further experiments.

Although the growth of *B. willdenowii* was less affected than that of *B. sterilis*, it was, like *B. sterilis*, most susceptible at the early post-emergence stages. Early (pre-emergence) and late (tillering) treatments failed to reduce growth below 100% and 72%

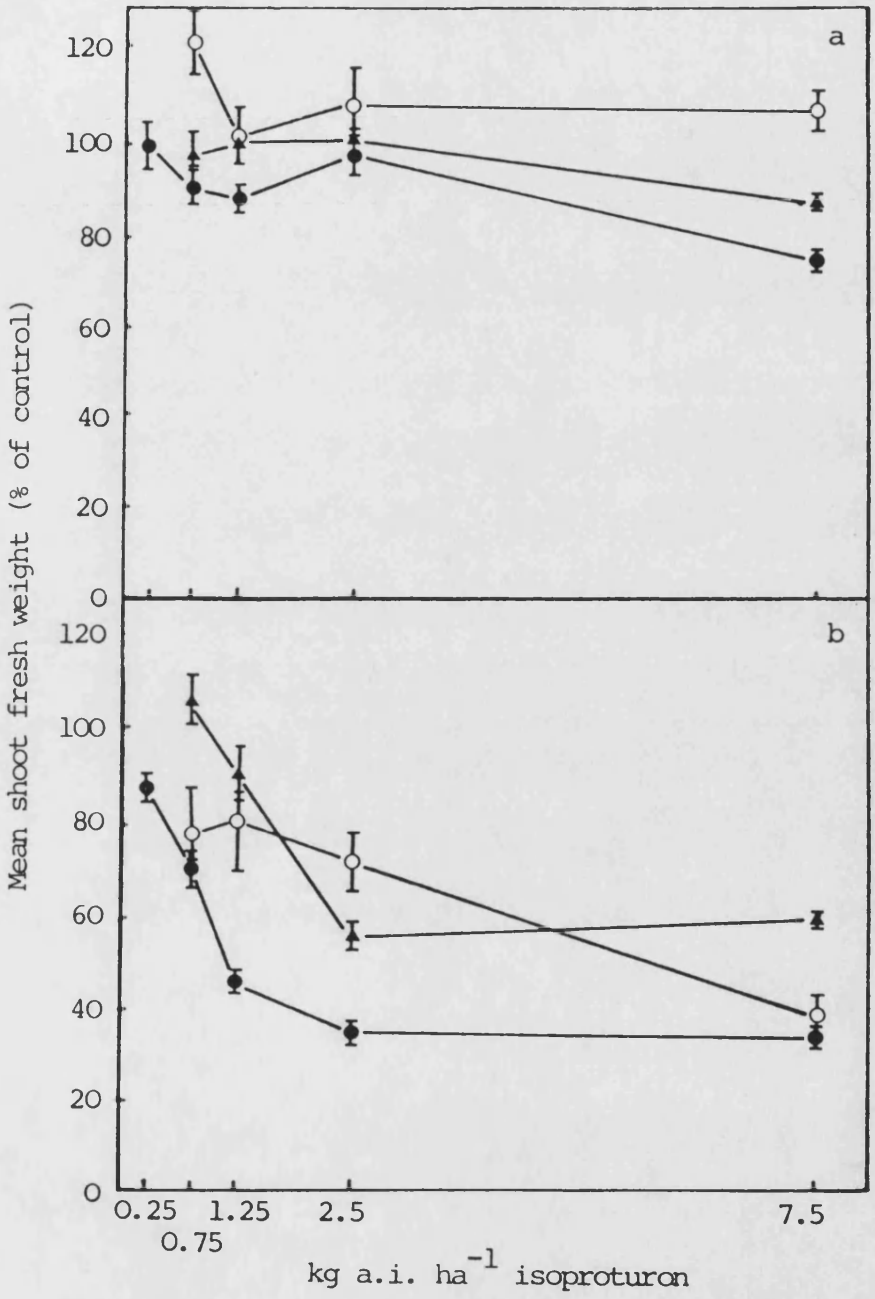


FIGURE 3.4

Shoot fresh weights following soil applications of several concentrations of isoproturon at a) the pre-emergence stage and b) the emergence stage. *B. sterilis* (●), *B. willdenowii* (O) and barley (▲).

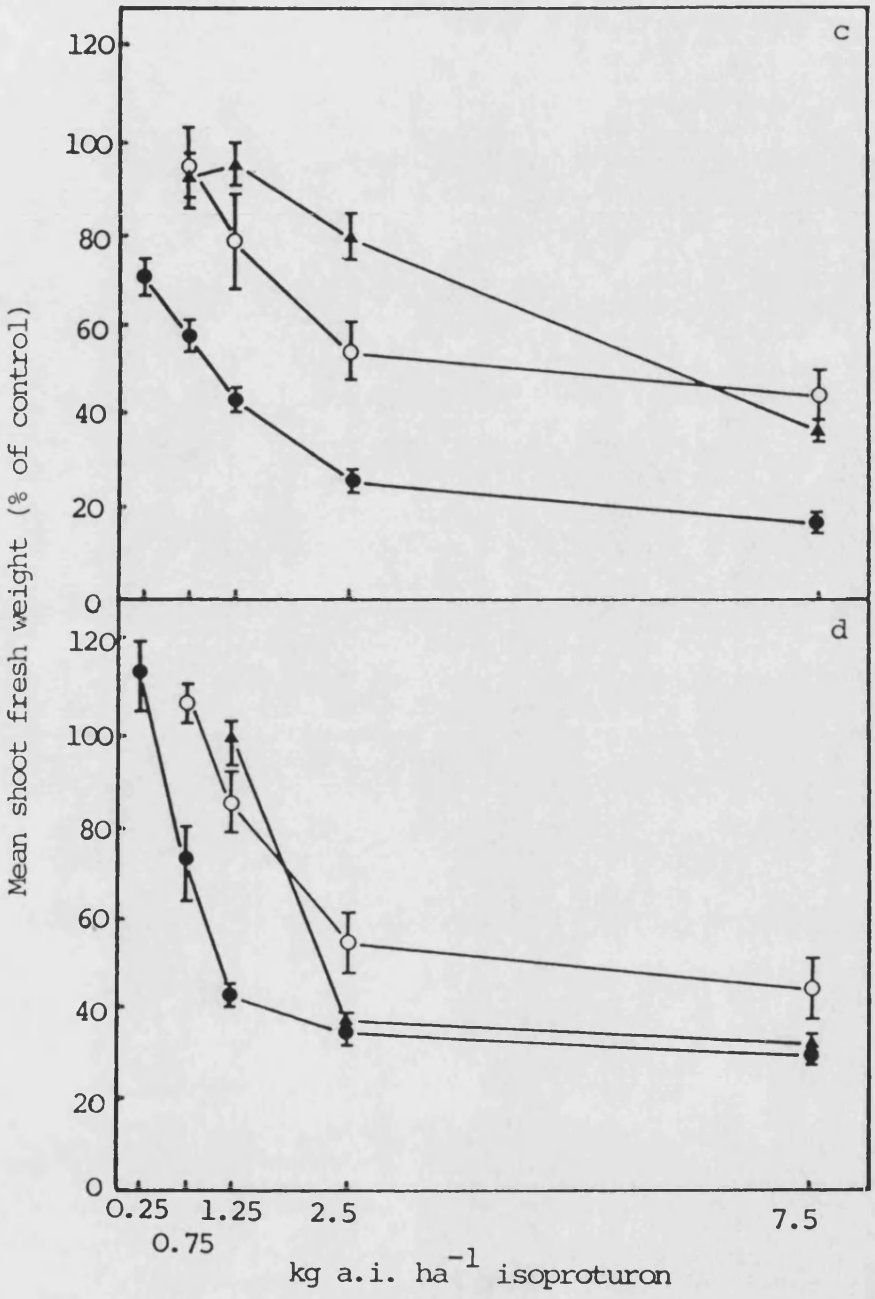


FIGURE 3.4

Shoot fresh weights following soil applications of several concentrations of isoproturon at c) the 1st leaf stage and d) the 2nd leaf stage. *B. sterilis* (●), *B. willdenowii* (O) and barley (▲).

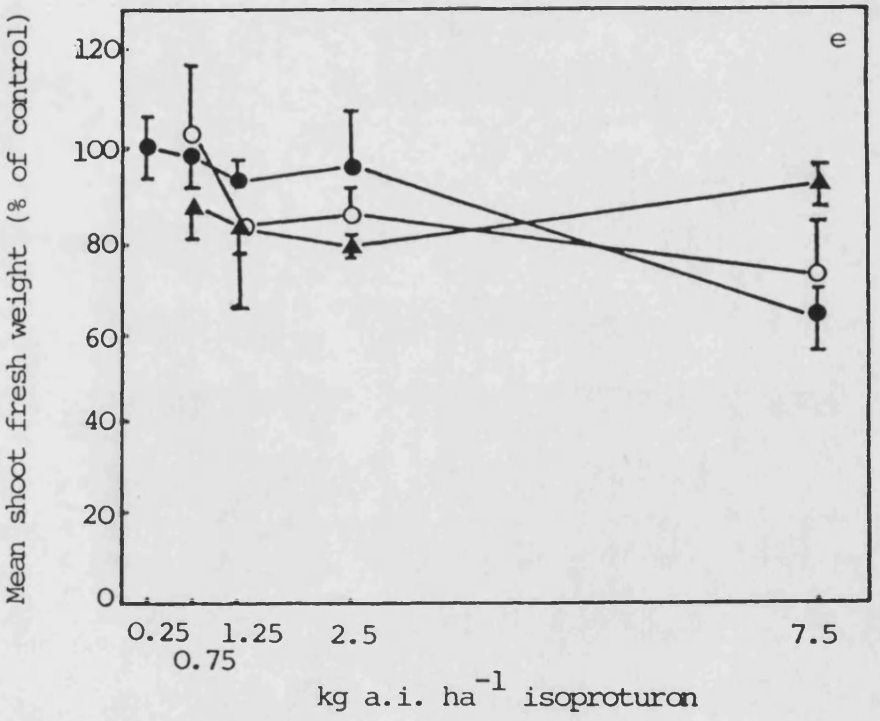


FIGURE 3.4

Shoot fresh weights following soil applications of several concentrations of isoproturon at e) the primary tillering stage.

B. sterilis (●), *B. willdenowii* (O) and barley (▲).

respectively (Figure 3.4 a and e). Albeit, poor control of *B. willdenowii* resulted from application of isoproturon at the most vulnerable 1st leaf stage. High rates did not substantially reduce growth of this grass species, and low rates actually led to significantly increased fresh weights of treated plants.

Barley showed more tolerance to isoproturon than either of the other species at the early post-emergence stages, where all three were at their most susceptible. However, at certain stages, *B. willdenowii* showed greater tolerance than the cereal. Nevertheless, isoproturon had a greater phytotoxic effect upon *B.sterilis* than barley at all stages except tillering. At rates below 2.5 kg a.i.ha^{-1}, fresh weights of barley plants were only slightly reduced (approx. 10%), but at higher rates, this figure reached 60 - 70% at some stages.

Analysis of variance indicated that at all stages except tillering, the three species were significantly different at P = 0.001 in their response to isoproturon. For all species, the concentration of the herbicide was significant at P = 0.001.

3.3.4 The effect of stage of growth on the phytotoxicity of isoproturon following foliar application

Reductions in fresh weight resulting from foliar application were substantially lower than from soil drench applications, though the stage of growth was again critical in determining the extent of damage. Unlike the case with soil drench treatments, species were only signficantly different at P = 0.001 at the pre-emergence stage. At every other stage, there were many interactions between species and concentrations, making it hard to be more precise.

Figure 3.5 a-d shows the effect of foliar application at various stages of growth. The largest margin of selectivity between species occurred at the 1st leaf stage (Figure 3.5 c), where *B. sterilis* was most susceptible and barley most tolerant to isoproturon. At every other stage, it was not obvious whether *B sterilis* or *B. willdenowii* was most susceptible, since the error of the mean was large. Fresh weights of treated barley plants were greater, but not significantly so, than those of control plants at almost every concentration and stage examined.

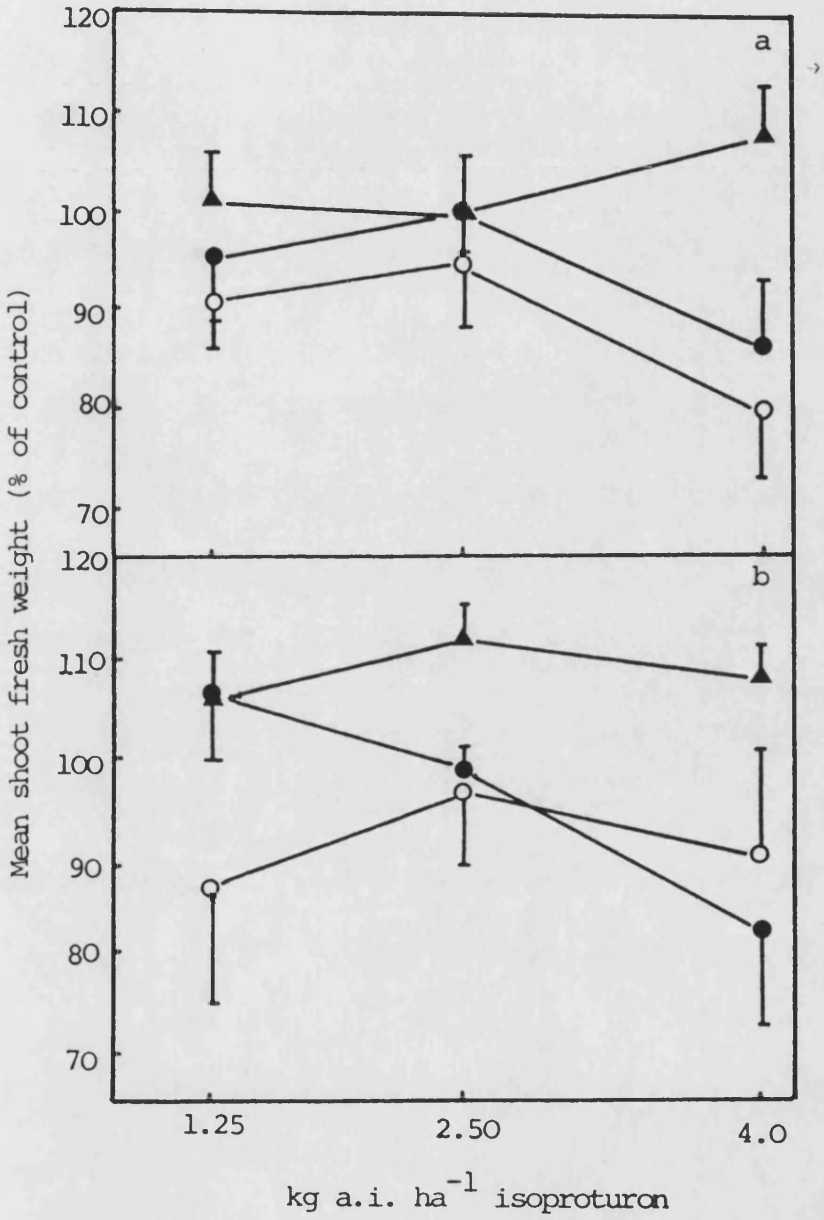


FIGURE 3.5

Shoot fresh weights following spray applications of several concentrations of isoproturon at a) the pre-emergence stage and b) the emergence stage. *B. sterilis* (●), *B. willdenowii* (○) and barley (▲).

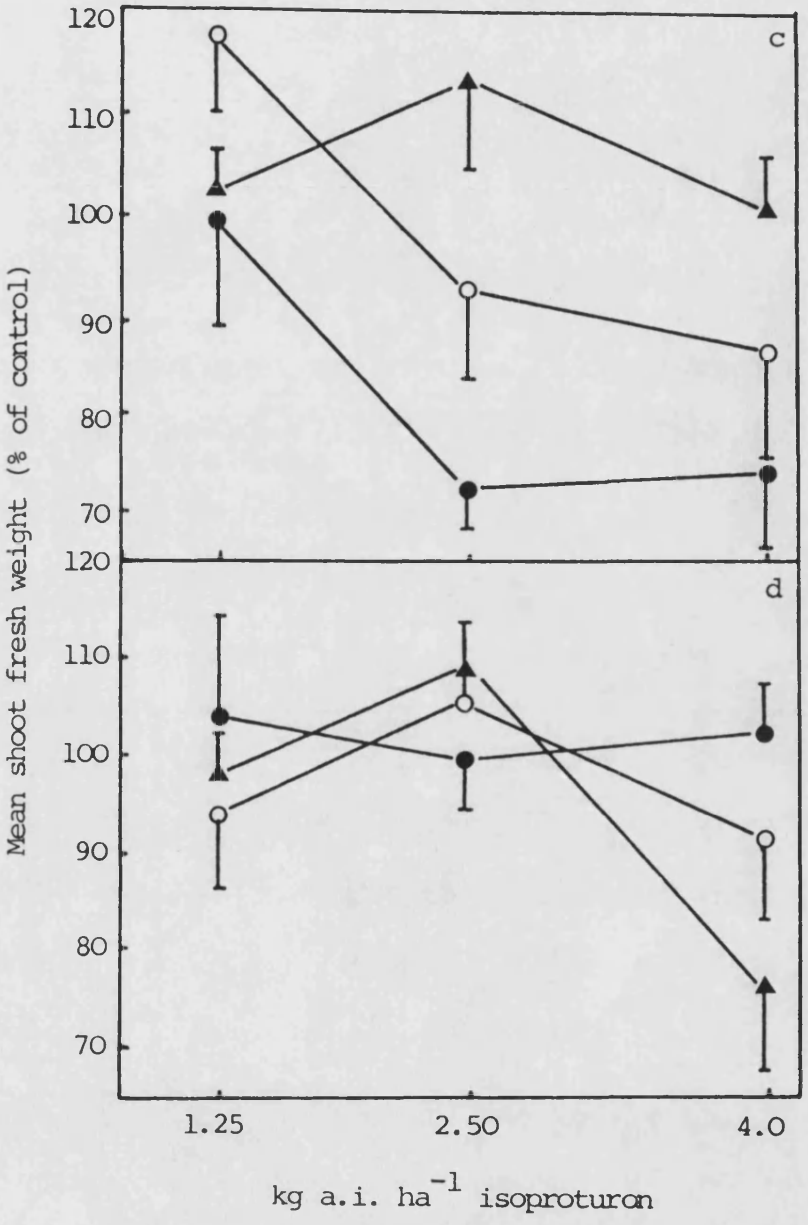


FIGURE 3.5

Shoot fresh weights following spray applications of several concentrations of isoproturon at c) the 1st leaf stage and d) the 2nd leaf stage. *B. sterilis* (●), *B. willdenowii* (○) and barley (▲).

3.4 DISCUSSION

The experiments in this investigation were designed to yield information which could account for the selectivity of isoproturon between different species, and to relate this to use in the field. The standardisation of materials and procedures employed was such that comparisons can justifiably be made between experiments. Controlled environments reduce the involvement of most of the variables that are encountered under field conditions, such as fluctuations in temperature, soil moisture and relative humidity. However, pot experiments are not directly comparable with the field situation. The volume of soil involved, and the movement of water and dissolved substances within the profile markedly differ. Subsequent plant growth may also differ, but pot trials are comparable with each other and results could, with caution, be extrapolated into the field.

The obvious heterogeneity between species makes comparisons more difficult, though problems have been avoided by expressing responses as a percentage of those of untreated plants of the same species.

i) The persistence of isoproturon in soils is poorly documented. Studies under laboratory conditions have yielded half-life values of 25 days in moist soil (F.C.) (Kulshrestha, 1983), though Fournier *et al.* (1981) recorded up to 85 days. Discrepancies can be related to differences in soil type and structure, and in the field, by variations in climatic conditions. Adsorption on to organic matter and drainage properties of the soil can alter activity (Ingram and Kyndt, 1981). Soil organic matter preferentially adsorbs most

herbicides (Hayes, 1970). However, adsorption is thought to become a serious problem only in soils with more than 10% organic matter content, and since John Innes No. 2 contains 9.5% organic matter, it was assumed that adsorption could be disregarded. Leaching of the herbicide below the depth of rooting could reduce persistence, but this is unlikely to occur in short duration pot trials.

Isoproturon was relatively stable in John Innes No. 2 over the 84 days test period, and this high degree of persistence must be a reflection of the degradative mechanisms. The initial degradative process of isoproturon in soil is dealkylation, which is usually mediated by soil micro-organisms (Kaufman and Kearney, 1976). Fournier *et al.* (1975) observed that degradation occurred mainly by microbial processes, though Kulshrestha (1983) reported that it was non-biological. Agents such as enzymes, inorganic minerals and water are important in this context (Crosby, 1976). A sterilised soil such as John Innes No. 2 would not be expected to support rapid biological breakdown.

The sensitivity of the bioassay procedure used in these experiments was such that a relatively high initial level of isoproturon was required, equivalent to 5 kg a.i.ha^{-1}. Experiments with other herbicides have shown that the amount applied has little effect upon the rate of subsequent decomposition in percentage terms. The rate of percentage loss from soil of simazine, for instance, was the same when the compound was applied to the soil surface at 0.56 kg a.i.ha^{-1} and at 2.24 kg a.i.ha^{-1} (Holly and Roberts, 1963). The evidence from this experiment suggests that isoproturon was sufficiently stable in John Innes No. 2 compost for breakdown during an experiment to be totally disregarded.

ii) The sensitivity or tolerance of a weed may be associated with the rapidity and extent to which its seed absorbs herbicides present in the soil solution (Reider *et al.*, 1970). Differential susceptibility of seeds to isoproturon was difficult to measure in soil studies, since there was no easy way to ensure that the herbicide was reaching the seed. By applying isoproturon to seeds in Petri-dishes, it was possible to obtain an indication of seed susceptibility, though the amounts reaching the seed were extremely high, far in excess of those likely to be encountered in the field.

Responses of the three species were significantly different at P = 0.001, indicating differential seed vulnerability to isoproturon. This may perhaps be associated with differences in seed size or seed coat thickness. Seeds of *B. willdenowii* were about half the size of those of *B. sterilis* when the lemma and palea were removed. Isoproturon may have had a greater effect upon *B. willdenowii* seeds because their small, flat shape ensured immersion under the solution in the Petri-dish. The more rounded seed of barley had a smaller surface area in contact with the solution, and its micropyle (pore for oxygen diffusion) may have remained above the solution level. Any of these factors could result in reduced water and herbicide uptake, though rates of these two do not always directly correlate. Herbicide uptake into soybean seed continued after water uptake had ceased (Reider *et al.*, 1970). However, the low percentage germination of untreated barley seeds suggests that moisture was perhaps limiting to this species under the experimental conditions imposed. Seed coat thickness is a less likely variable producing differences in susceptibility, since *B. sterilis* seeds showed a similar response to isoproturon as those

of barley. Barley seeds possess tough husks, perhaps making penetration difficult. Lemmas of the *Bromus* spp. are thin and papery, minutely rough and 7-nerved (Hubbard, 1968). Since they do not vary widely between species in shape and thickness, they cannot account for the differential uptake between *B. sterilis* and *B. willdenowii*. While imbibition and uptake rates can vary between species, they are probably not limiting in an experiment lasting as long as ten days. It is not clear, however, whether the susceptible *B. willdenowii* seeds simply absorbed more isoproturon, or whether they exhibited a more extreme reaction to the herbicide that did actually penetrate the seed coat.

The damage caused by isoproturon to seeds is unlikely to interfere with the same processes as are affected in seedlings, since the photosynthetic apparatus has not yet developed. Isoproturon may have detrimental effects upon the membranes and enzymes present in seeds, or the formulation may contain ingredients toxic to certain mechanisms. Gray and Weierich (1969) showed that herbicides absorbed by physical processes during early imbibition caused an inhibition of seed metabolism. Pusztai and Vegh (1978) noticed that some herbicides caused mutations in barley seeds. The enhanced damage to seeds of all three species caused by continuous, high intensity light cannot be explained by phytochrome-mediated responses, since untreated seeds were not markedly altered by light. A photo-induced breakdown of the herbicide to a more toxic product would seem a possibility were there any evidence that isoproturon was photo-sensitive. However, its stability to light is well-documented, so another explanation is suggested.

The seeds that successfully germinated in isoproturon treated dishes produced poorly developed seedlings, possessing shorter and weaker radicles and plumules than untreated seedlings. This suggests that isoproturon was not available in sufficient quantities to prevent germination, but could retard growth after the food reserves in the endosperm were consumed. Pestemer (1976), using monolinuron, found no effect on germination during the first 5 days after sowing cress seeds, but noticed a retardation once reserves were consumed. The herbicide may perhaps prevent the development of the photosynthetic apparatus.

iii) Isoproturon is generally classified as a translocated and soil-acting herbicide (Anon, 1984c); the experiments reported here also suggest that soil activity is more important, since root drenching was more toxic to all three species than foliar spraying. Similar findings were reported by Richardson *et al.* (1977) and Okereke *et al.* (1981b) for *B. sterilis* plants and by Blair (1978) for blackgrass. They suggested that there was little activity as a result of foliar uptake, when comparing an overall spray with one in which the soil was protected at spraying by perlite. In the present study, the soil surface was exposed to the spray hence some soil activity would be expected, but the effects were still minimal compared with a soil drench. The greater response of soil drenching may relate to the larger volume of carrier, in this case water, applied with isoproturon. A herbicide may be almost inactive if it remains on the soil surface (Riley and Morrod, 1976). Moist soils are essential for good herbicidal activity (Ingram and Kyndt, 1981), and a large volume rate will help to ensure thorough distribution

within the profile. Since plant size at the early post-emergence stages is small, the majority of herbicide will reach the soil directly upon spraying. It would seem that the reason for poorer plant response to spraying is associated with the lower volume rate. In the field, it would be highly impractical to use large volumes of carrier liquid, and in any case, low volume spraying is successful, though factors such as rewetting of foliage by dew and heavy rainfall must enhance herbicide activity.

The results presented here suggest that, with the use of relatively larger concentrations of isoproturon than are required for drenching, a similar level of control can be achieved with spraying. Though the site of herbicide uptake can vary between species, there was no indication that either soil or foliage uptake was preferential in a particular species. The site of uptake for each species will be examined in a later section. A more detailed study of the leaf surface characteristics may explain why foliage applications result in poorer control.

The standardisation of soil moisture content throughout the experiment guaranteed a similar water content of the plants at harvest. This was the justification for assessing damage to the plants in terms of fresh weights in preference to dry weights. Shoot growth is often reduced before the appearance of injury symptoms, so that fresh weight can be used as a rapid indication of herbicide damage. A treatment period of ten days was chosen since any shorter period did not allow for the expression of substantial differences between treated and untreated plants. Ingram and Kyndt (1981) measured chlorosis of blackgrass leaves within a few days of treatment, and plant death within 14 - 42 days, depending on

temperature. Phytotoxicity effects are obviously greater following longer treatment periods, however young plants susceptible to isoproturon may perish before the harvesting date. It must be noted that these experiments were designed to reveal the margin of selectivity between species, not to produce the maximum herbicidal effect.

iv) The stage of growth of plants at treatment has often been shown to have a marked effect upon susceptibility to herbicides. A herbicide may only give a sufficient degree of control when applied at a particular growth stage of the weed, for example, metoxuron gave effective post-emergence but not pre-emergence control of *B. sterilis* in pot trials (Ayres and Richardson, 1981). A similar response of *B. sterilis* to isoproturon was apparent in the present study, considerable tolerance being exhibited until the emergence stage. Reasons for the tolerance of *B. sterilis* at the pre-emergence stage are unclear, but may be related to the seed properties discussed in the previous experiment. However, seed properties fail to explain why *B. willdenowii* seeds were extremely tolerant at the pre-emergence stage in soil, when they were the most susceptible in Petri-dish tests. Perhaps it is too presumptuous to assume that isoproturon is constantly available in the soil solution for seed uptake, and indeed that seed response is related solely to uptake. There may be more complex interactions involved, such as efficient herbicide metabolism in *B. willdenowii*, and these possibilities will be investigated later. Hance and McKone (1976) reported that the earliest useful stage at which to apply photosynthetic inhibitor herbicides is probably dependent upon the time taken to exhaust the

food reserves of the seed, as only then do compounds produce large effects on plant growth. It is possible that isoproturon cannot be absorbed in large quantities until the seedling roots have developed sufficiently. A treatment period of only ten days may be too short for effects upon plant size to become obvious.

The performance of isoproturon was greatly improved when it was applied at the early post-emergence stages, an observation which has also been made in the field for other annual weeds (Hubbard *et al.*, 1976; Cussans *et al.*, 1982). The greatest margin of selectivity between the three species occurred at the 1st leaf stage, suggesting that applications at this stage would result in minimal crop damage coupled with maximum control of the weed. Some degree of crop damage may have to be accepted in the field, but it can be kept to a minimum if isoproturon is applied before the most vulnerable stage of the crop, which appears to be 2 - 3 leaves unfolded (Zadoks GS 13) (Tottman *et al.*, 1975). The results reported here support the findings of Tottman and co-workers in that barley was most susceptible at the 2nd leaf stage.

All three species became relatively tolerant of isoproturon after the 3 leaf stage, an observation also made by Palmer (1981) for *B. sterilis* in the field. Applications at the early tillering stages cause a variable response of *B. sterilis* in the field (Redbond, 1980). The suggestion that tolerance increases with plant age is supported by the results of both soil and foliar applications. In the case of soil-applied herbicides, it is likely that a decrease in the tissue concentration occurs as the plant increases in size, as proposed by various workers (Hagimoto and Yoshikawa, 1972; Macquarrie *et al.*, 1985). Root size will also

increase with plant age, with the major absorption zone growing beyond that of herbicide activity. With respect to foliar applications, leaf surface characteristics may alter with age. Blackman *et al.* (1958) recorded an increase in retention as barley passed the 3 leaf stage, due to a less upright growth habit. Kirkwood (1972) and Leon and Bukovac (1978) recorded changes in the cuticle wax barrier with age, resulting in a reduction in absorption. Trichomes may change in number, size and structure as the leaf matures (Åberg and Steckó, 1976), altering leaf wettability. These factors will be examined individually in subsequent sections to assess their contribution to selectivity.

4. HERBICIDE RETENTION

4.1 INTRODUCTION

Leaves are covered by a lipoidal, non-cellular, non-living membrane called the cuticle (Bukovac, 1976). It is formed of materials synthesised in the epidermal cells and subsequently extruded to the surface. The cuticle has four layers, namely an outer epicuticular wax, cutin, pectin and an innermost region of cellulose, which is actually the periclinal wall of the epidermal cells (Sitte and Rennier, 1963). The cutin, a non-cellular layer composed of polymerised long-chain fatty acids, alcohols (Norris and Bukovac, 1968), dibasic and hydroxy carboxylic acids (Hartley and Graham-Bryce, 1980), has both hydrophilic and hydrophobic properties (van Overbeek, 1956), the latter being predominant (Hartley and Graham-Bryce, 1980). The pectins, which are likely to be composed largely of polyurenoides, and the cellulose epidermal cell wall are both hydrophilic (Crafts and Foy, 1962). The epicuticular waxes consist mostly of n-alkanes, straight-chain saturated ketones and alcohols, and carboxylic esters (Hartley and Graham-Bryce, 1980). Epicuticular wax development and its structure and composition is important in controlling the responses of many plant species to post-emergence herbicides (Bukovac, 1976; Holly, 1976; Baker, 1980). Wax particles occur in a variety of forms and are probably the most important barrier to herbicides (Robertson *et al.*, 1971). The physical form of this wax layer determines the degree of water reflection, while its chemical nature controls selective permeability of compounds.

The exact pathways involved in cuticle penetration are still subject to conjecture, but it is generally believed that non-polar

compounds follow a lipoidal route and polar materials an aqueous route (Crafts, 1956). Penetration is thought to be a physical process which is directly influenced by a number of factors, including the chemical structure and polarity of the penetrating compound (Sargent, 1976) and the hydrophilic/lipophilic balance of any surfactant (McIntosh *et al.*, 1981). Surfactants are added to many herbicide formulations to facilitate handling and/or application. Their function is to reduce surface tension, and this can occur at concentrations as low as 0.01%, remaining more or less constant above 0.1% (Jansen, 1973). Surfactants can, in this way, increase retention of sprays, yet they tend to reduce selectivity (Ayres and Richardson, 1981), since high concentrations can sometimes dissolve wax or cuticle components and change their physicochemical properties (Kirkwood, 1977). The inclusion of 'Tween 20' (polyoxyethylene sorbitan monolaurate) in test compounds is common, since it is chemically relatively inert due to its lack of ionisation.

The nature of the leaf surface is only one of many factors which can influence the amount of spray retained. Penetration may occur largely by preferential sites, including stomata, guard cells and the basal cells of trichomes (Sargent and Blackman, 1962). While the role of the stomata is still controversial, it has been argued that they do facilitate herbicide entry, since spray solutions under certain conditions may move in mass through the stomatal pore (Dybing and Currier, 1961; Greene and Bukovac, 1974). The sub-stomatal cavities are generally lined with an extension of the cuticle, but it is much thinner in these areas, probably offering little resistance to the passage of small molecules (Hartley and

Graham-Bryce, 1980). Other workers believe that preferential penetration of the guard cells is more probable than entry via the pore (Sargent and Blackman, 1962). Ectodesmata, minute channels from the interior of the epidermal cells which appear to terminate below the cutin, are particularly numerous over guard cells (Franke, 1967). However, their role, and indeed their existence is still a contential issue. Nevertheless, Veerasekaran *et al.* (1977) recorded greater uptake of asulam through the stomatal-bearing abaxial surface of bracken *(Pteridium* spp.), suggesting that these sites may be important in penetration. When leaves have been wetted, the base of trichomes often retain a ring of water after the plane surface between trichomes has drained. The cuticle surrounding trichomes will have a greater residual density of applied herbicide than other regions (Hartley and Graham-Bryce, 1980). The pattern and abundance of trichomes on each surface determine leaf wettability and may change as the leaf matures (Åberg and Steckó, 1976). Both stomata and trichome numbers may vary between adaxial and abaxial surfaces of a particular species, and between species themselves.

Leaf orientation and angle will influence spray interception (Bukovac, 1976), the surface exposed to the spray being important in determining retention. The horizontally projected leaf area has been used as a crude quantitative estimate of retention for comparison between species (Davies *et al.*, 1967). The retention of a non-toxic dye on the plant surface gives a more accurate measure of herbicide availability for penetration.

Comparative retention by the three species probably plays an important part in determining their relative susceptibility to isoproturon. The leaf surfaces of the three species were examined

using a scanning electron microscope to determine any distinct visible differences between them. Photographs were taken at magnifications of 150 and 6,000 to show the frequency and arrangement of cells, trichomes and stomata and the size of wax particles respectively. Plants were sprayed with a water-soluble dye to evaluate the degree of retention on leaf surfaces, and to assess its contribution to selectivity.

4.2 MATERIALS AND METHODS

4.2.1 Scanning electron microscopy

Plants were grown for 14 days in soil maintained at 75% pot capacity throughout. Strips of leaf tissue, 2 cm long, taken from the middle of the 1st leaf, were coated in liquid nitrogen and freeze-dried for 24 hours at -60°C in an 'Edwards Pearse EPD3' tissue dryer, using phosphorus pentoxide as a drying agent. Each strip was glued, with the relevant surface uppermost, to a planchette using a carbon-based glue. Each planchette was coated in gold in an 'Edwards S 150B' sputter coater. When dry, planchettes were individually examined in a 'JEOL-JSM-35C Stereoscan S4-10' scanning microscope at KV 15 and a working distance of 39. An internal camera (Mamiya) was used to photograph the images at magnifications of 150 and 6,000. Film was developed for 10 min in 'Kodalith super RT' developer and fixed in 'Kodafix' solution at a ratio of 1:3 with water. Prints were made from the negatives using 'Kodak' paper developer and 'Kodafix' at 1:7 with water.

4.2.2 Dye retention

Soil-grown 14 days old plants were thinned down to one per pot, and sprayed with a 2% w/v acid red dye solution (disodium salt of 8-acetamido-2-phenylazo-1-naphthol-3,6-disulphonic acid), containing 0.1% v/v 'Tween 20' (polyoxyethylene sorbitan monolaurate) wetting agent. The volume rate of the solution was 600 ℓ ha^{-1} applied through a 'Teejet 80015 LP' nozzle attached to a hydraulic sprayer (described previously). Plants were left to dry for 30 min before returning them to growth cabinets.

After 24 hours, the shoots were excised at soil level and weighed. Each shoot was rinsed for 2 min in 10 cm^3 of distilled water containing 0.01% v/v 'Tween 20'. The optical density of each solution was measured at 540 nm on a 'Shimadzu UV-260' UV-visible recording spectrophotometer. (A visible light absorbance spectrum showed 540 nm to be the maximum absorbance wavelength for acid red.) The concentration of dye retained by each plant was calculated by making a range of dilution standards of acid red and recording their optical density, thus producing a calibration curve. Acid red had previously been tested for light-fastness, relationship of concentration to optical density, and ease of recovery from plant surfaces (Hibbitt, 1969).

Leaf surface areas of each species were measured at 14 days using a 'LI-COR, LI 3000'portable area meter (Crump Scientific Products Ltd., Rayleigh, U.K.).

4.3 RESULTS

4.3.1 Leaf surface morphology

Plate 4.1 A-C show the adaxial surface of leaves of *B sterilis*, *B. willdenowii* and barley respectively at x 150. Trichomes were abundant on the leaf surface of *B. sterilis*, being long and often spiralled. Those on the surface of *B. wildenowii* seedlings were shorter and needle-like, and equally abundant. Few such large trichomes were present on barley leaves, though this species, and to a lesser extent the *Bromus* spp., possessed minute projections along the edge of the leaf, similar in appearance to rose thorns.

The abaxial surfaces of the three species are presented in Plate 4.2 A-C, the species in the same order as above. Trichomes were more abundant on the lower leaf surface of *B. sterilis* than on the upper surface. In this species, the form and size of trichomes was similar to those present upon the adaxial surface. This observation also applied to *B. willdenowii*, though trichomes were not more abundant on this surface. Barley again possessed few long hairs on the leaf blades, but the edges were covered in thorn-like projections. On neither surface were there apparent visible differences in stomatal size or frequency between species.

Leaf surfaces were magnified x 6,000 to examine wax particles (Plate 4.3 A-C). There were dense networks of small crystalline wax plates projecting from the surface of all three species. These wax crystals appeared similar in shape and arrangement, though they differed in size between species. The platelets of barley leaves were larger and appeared to be arranged more densely. There were no distinct differences between adaxial and abaxial surface waxes (abaxial not shown).

PLATE 4.1

Leaf surface morphology. Adaxial surfaces at x 150 of A) *B. sterilis*, B) *B. willdenowii* and C) barley.

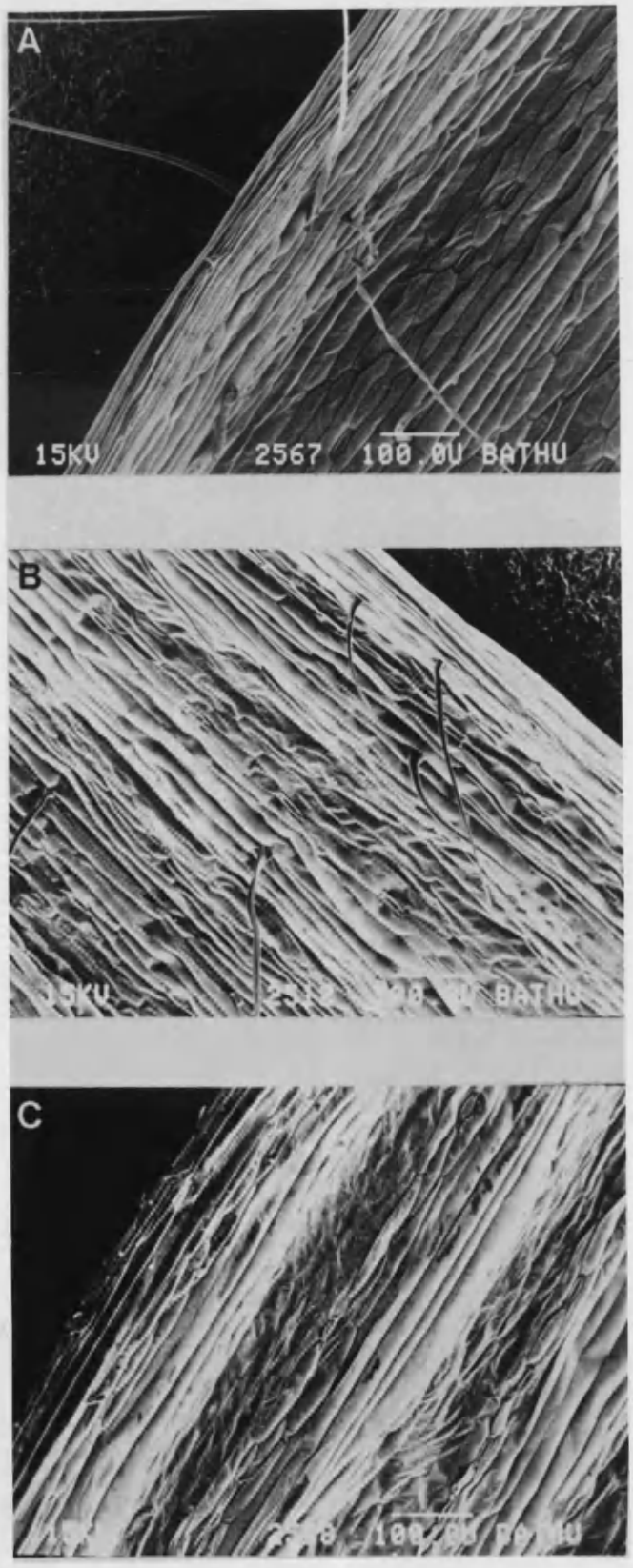

A
15KU 2567 100.0U BATHU
B
C

PLATE 4.2

Leaf surface morphology. Abaxial surfaces at x 150 of A) *B. sterilis*, B) *B. willdenowii* and C) barley.

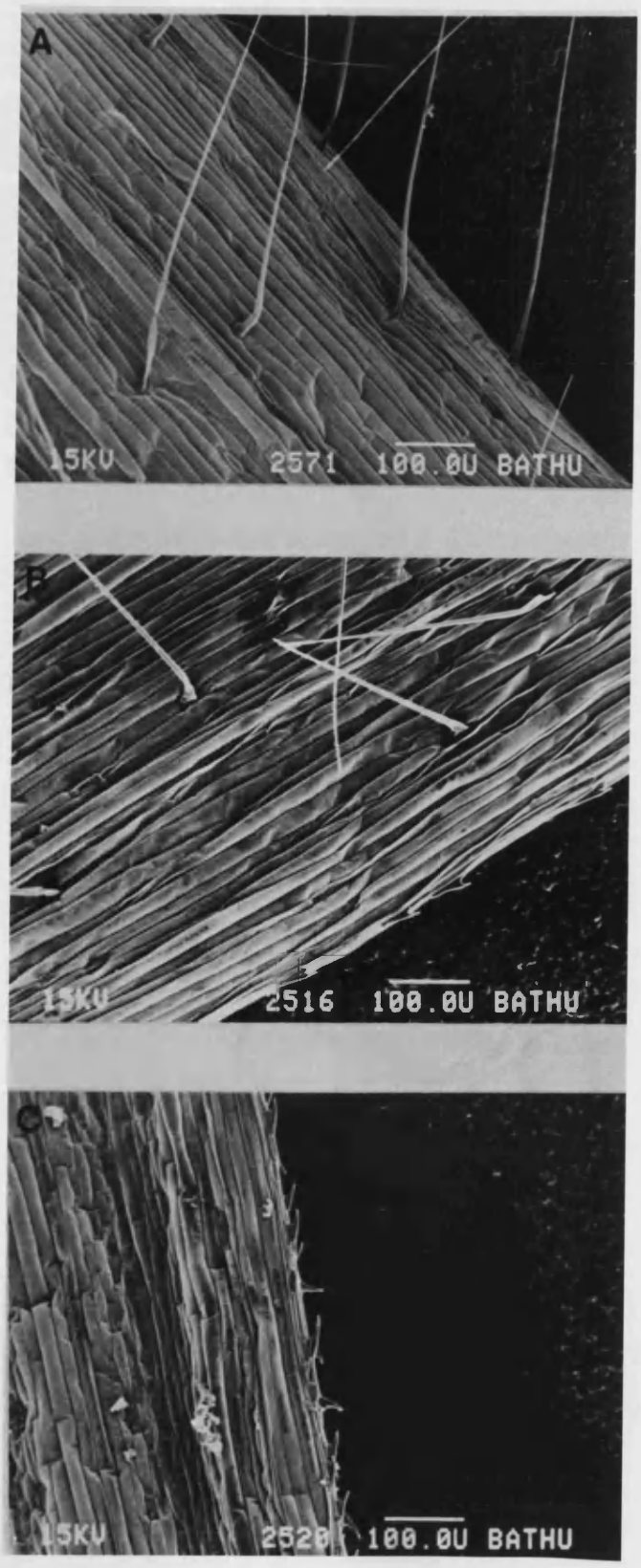
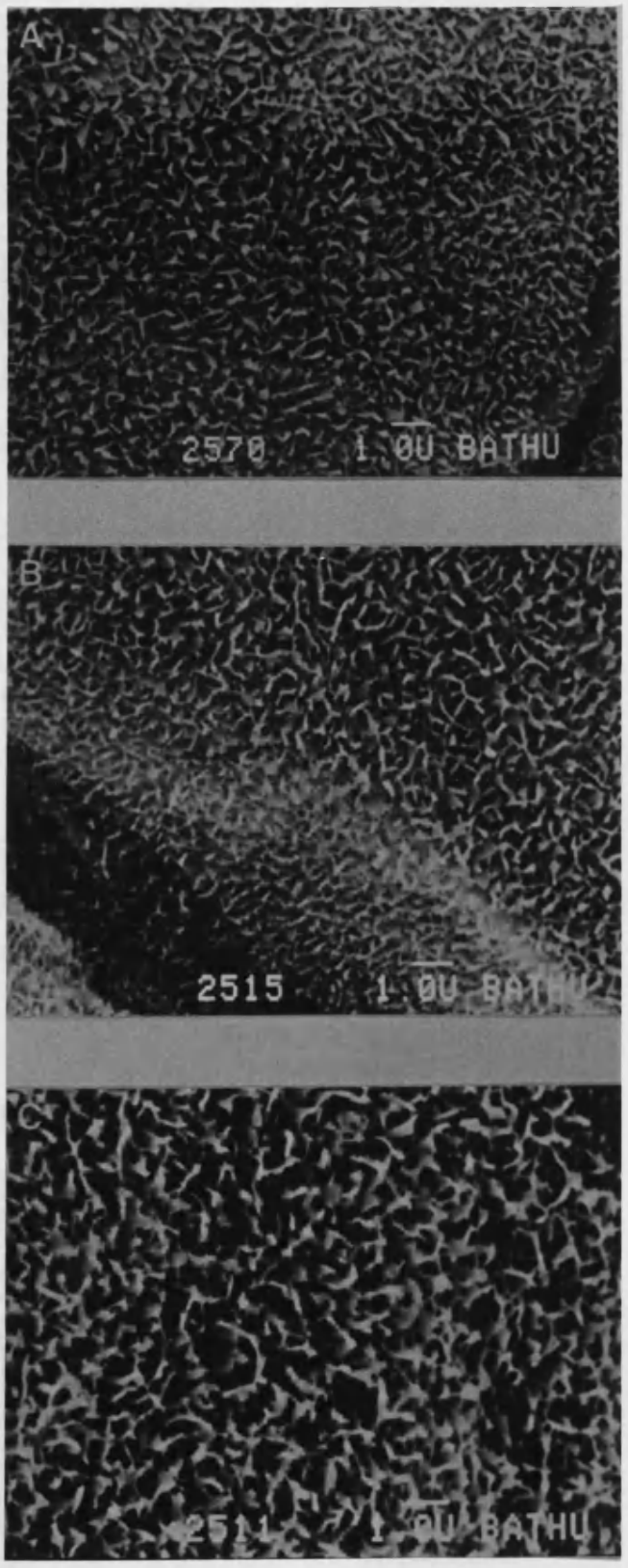

A
15KV 2571 100.0U BATHU
B
15KV 2516 100.0U BATHU
C
15KV 2520 100.0U BATHU

PLATE 4.3

Leaf surface morphology. Adaxial surfaces at x 6000, mid-lamina region of A) *B. sterilis*, B) *B. willdenowii* and C) barley.


A
2570 1 0U BATHU
B
2515 1 0U BAT
C
2511 1 0U BATHU

4.3.2 Spray retention characteristics

Table 4.1 shows the quantities of dye retained per unit weight of leaf tissue and per unit leaf area of *B. sterilis*, *B. willdenowii* and barley. *B. sterilis* retained the smallest, and *B. willdenowii* the largest amount of dye per unit weight and leaf area, the value for the latter species being more than double that of the former. Barley leaves were intermediate in their response to dye retention. Species response was significantly different at P = 0.001.

TABLE 4.1

Dye retention (μ g mg FW^{-1} and μ g cm^{-2}) of 14 day old plants of *B. sterilis*, *B. willdenowii* and barley.

species	μ g mg FW^{-1}	μ g cm^{-2}
B. sterilis	2.66 (0.12)	128.5 (4.5)
B. willdenowii	9.11 (0.26)	256.9 (6.9)
barley	4.12 (0.63)	173.5 (5.6)

4.4 DISCUSSION

Blackman *et al.* (1958) demonstrated that differential retention played a major role in the selectivity of the substituted phenoxyacetic acid herbicides. Differential retention of a water-soluble dye was apparent in the present study, though results must be interpreted with some caution since the behaviour of a suspension of herbicide in water may be different from that of an aqueous solution of dye (Verity *et al.*, 1981). For the purpose of comparison between species, it was assumed that the dye simulated the herbicide with respect to retention on leaf surfaces. This assumption was justified by the addition of a non-ionic surfactant to the dye solution, such as would be present in the commercial formulation of isoproturon.

Leaf angle and orientation often play an important part in determining the relative susceptibility of different species to a particular herbicide (Bukovac, 1976). In general, the more vertical the leaf, the less spray will be retained (Davies *et al.*, 1967). There is a greater likelihood of a falling drop being reflected from a leaf if the angle between the droplet path and the leaf surface is small i.e. a high angle of incidence. Differences in retention related to orientation are only expected to be large between plants with differing growth habits. The three monocotyledonous grasses concerned here all have an essentially similar erect, upright growth habit, characteristic of young grass seedlings. However, the horizontally projected leaf area was larger in *B. willdenowii* because these plants were taller at 14 days than the other species, and the longer leaves had begun to droop from the near vertical. Increased retention occurs on drooping leaf tips, a feature which,

in the field, is also accentuated by the air stream from the sprayer (Davies *et al.*, 1967). Though fresh weights of barley plants were greater than *B. willdenowii*, the leaf surface area : volume ratio was smaller, i.e. 10 mg of barley leaf tissue covered a smaller area than the same weight of *B. willdenowii* tissue. Relative weights of stem and leaf tissue may be important in determining retention, since blades are more horizontal than sheaths. Barley leaves are also supported by a thicker, more erect leaf/sheath than the other species. *B. sterilis*, although possessing a high capacity for quick growth (Grime, 1979), had a slower relative growth rate than barley at the early stages (Gray, 1981). Its small size at 14 days may partially explain the poor retention on leaf surfaces. By expressing retention as a function of both fresh weight and leaf surface area, discrepancies relating to plant size should be avoided. However, the amount of herbicide retained per unit weight of tissue is most important in terms of toxicity to the plant (Blackman *et al.*, 1958).

Differences in leaf surface characteristics between species are a likely cause of differential response to dye retention. If stomata or guard cells are indeed preferential sites of entry, the relative numbers of stomata on the leaf surface could influence herbicide retention and penetration. However, there were no apparent differences among species in the frequency of stomata. Hairiness does not necessarily indicate poor retention, but if hairs are both rigid and water-repellent, they can be difficult to wet (Roberts, 1982). There is evidence that water retention is greater on leaves with an 'open' as opposed to a 'closed' trichome pattern (Challen, 1962). The open pattern enhances wetting due to capillary action, whereas the closed pattern depresses it by trapping air beneath the

spray droplets. Neither the rigidity nor the abundance of trichomes on the adaxial surface of *B. sterilis* leaves appeared sufficiently extreme to account for poor retention (Plate 4.1 A). However, in such an erect, twisted leaf, spray interception is likely to occur on both surfaces, thus the abundance and more closed pattern of trichomes on the abaxial surface may have reduced retention. Froud-Williams *et al.* (1980) suggested that the hairy surface of *B.sterilis* leaves accounted for poor retention by this species. Since barley leaves, possessing few hairs, showed poor dye retention, spray interception must be related to factors other than the number of trichomes.

Low retention of dye on barley leaves may perhaps be explained by the nature and arrangement of wax particles. Epicuticular wax has been shown to constitute a barrier to the foliar entry of some herbicides into plants (Hunt and Baker, 1982; Whitehouse *et al.*, 1982). Barley is reported to have a water-repellent cuticle, since the wax particles are arranged in such a way that runoff occurs (Blackman *et al.*, 1958). There were no apparent differences in the structure and arrangement of the wax cuticles of barley and the *Bromus* spp. in this study (Plates 4.3 A-C). McIntosh *et al.* (1981) similarly found no obvious structural differences in the cuticle waxes of wheat, blackgrass and wild oat leaves. Although there appeared to be no structural differences, variations in the size of wax platelets were evident. Barley cuticle waxes were equally densely packed, but the individual crystals were larger than those of the *Bromus* spp. However,unless the larger size of platelets reflects a thicker epicuticular wax layer, this is unlikely to reduce retention. In any case, surfactants within the formulation, in

addition to reducing the surface tension of spray solutions, tend to dissolve wax deposits on leaves (Price, 1977). McIntosh *et al.* (1981) observed that the wax layers on wheat leaves were destroyed following application of isoproturon plus 0.2% surfactant. In the current study, the non-ionic surfactant 'Tween 20' present in the dye solution probably lowered the surface tension, thus improving the retention and spreading of droplets on plant surfaces. In addition, it may have partially dissolved the waxes, nevertheless 'Tween 20' has no major independent action other than influencing retention (Davies *et al.*, 1967). It is assumed that the surfactant had a similar effect upon the waxes of all three species, though instances of surfactant-species interactions have been recorded. For example, Midgley (1982) found that the activity of MCPA and dichlorprop against clover was increased but against chickweed it was decreased with the addition of a surfactant. These variations may relate to differences in the chemical composition or physical state of the epicuticular waxes, and may provide a basis for selectivity in some instances. However, it is unlikely that the epicuticular wax structure accounted for selective retention in this study. Differences in trichome numbers, or perhaps leaf angle, are more likely explanations of the differential retention of sprays by *B. sterilis*, *B. willdenowii* and barley that was observed.

5. HERBICIDE UPTAKE

5.1 INTRODUCTION

Many external physical processes may influence the extent to which the active ingredients of soil-applied herbicides reach the root surface. Herbicide molecules can be transferred from the soil surface to the rooting zone via water and air-phase diffusion, leaching and dynamic dispersion (Hartley, 1976). The efficiency of this transfer will depend upon the pore size distribution of soil, but more importantly, the soil moisture content, and it is generally agreed that moist soils are essential for good herbicide activity (Ingram and Kyndt, 1981). Adsorption of the herbicide molecule on to soil colloids will generally reduce herbicidal activity, though it may extend the period over which the herbicide is effective. The solubility of the compound itself will influence both the effectiveness and rate of movement through the soil, although the total quantity added to the soil is so small compared with the relatively large volume of soil water, that it is not the all-important factor that some authors suggest.

Root absorption appears to take place primarily through root hairs, located just behind the root apical meristem, since the area 5 - 50 mm behind the root tip has been identified as the primary site of entry (Tanton and Crowdy, 1972). Dissolved substances may diffuse unrestricted via the apoplast (cell walls) through the cortex to the endodermis. At this point, herbicides must enter the symplast, due to the presence of the Casparian strip. It is also possible that sufficiently fat-soluble molecules might diffuse through this suberized layer and enter the stele. Once in the stele, long distance transport is possible.

In addition to root absorption, a considerable number of soil-acting herbicides have been shown to enter some kinds of seedlings through the sub-surface shoot (Parker, 1966; Nishimoto *et al.*, 1967; Prendeville *et al.*, 1967). All cited reports of uptake via the sub-surface shoot have concerned emerging seedlings following pre-emergence application. Blair (1978) reported that root uptake into annual grasses was more important for urea herbicides at both pre- and post-emergence stages. Phytotoxicity will depend upon the position of the roots and growing point in relation to the site of herbicide placement (Rogers and Funderburk, 1967). In the field, the farmer can control crop seed depth at planting to achieve "depth protection" from some herbicides (Gerber and Guth, 1975). However, if selectivity depends on planting depth, herbicide movement in the soil is important. Addala *et al.* (1985) stressed the risks involved in "depth protection" strategies if the crop has no measure of tolerance, since low concentrations may damage a sensitive plant yet not show up in a leaching study.

Leaching does not necessarily occur uniformly in soil, thus availability of the herbicide to the developing seedlings is conjectural. Herbicide presentation to the roots in nutrient solution ensures its availability so that uptake and translocation can be measured directly (Sargent, 1976). Deleterious effects following uptake of a herbicide can be identified by measuring plant weights and pigment contents. Damage to the photosynthetic apparatus is indicated by reduced chlorophyll contents, since chlorophyll degradation follows carotenoid breakdown (Pallett and Dodge, 1980). As pigment levels decreased, ethane generation, an

indicator of membrane breakdown (Reily *et al.*, 1974) increased. Selectivity between species relating to uptake cannot be distinguished on the basis of these results, since other factors may contribute to the observed effect. The use of radiolabelled tracers in herbicide research has allowed previously inextricable factors to be differentiated. Uptake rates can vary between species under similar conditions, and are partially dependent upon plant size and root distribution (Sargent, 1976). Many workers have shown that uptake of a herbicide from nutrient solution by intact plants decreases from an initial rapid rate to a much slower rate sustained for much longer periods (Shone and Wood, 1973; Walker and Featherstone, 1973). In the case of ion uptake, the first phase is thought to be a passive one, and the second an active process requiring metabolic energy. Although controversial, the bulk of evidence for herbicides suggests that metabolic processes are not involved in uptake by roots. The general picture to emerge from uptake studies is a rapid initial accumulation, greater than can be accounted for by water uptake alone, and presumably reflecting diffusion and partition on to the surfaces of the free space, followed by a slower further uptake reflecting movement to the less accessible tissues of the root and possibly passage across membranes (Hartley and Graham-Bryce, 1980). Membrane permeability could be of importance in determining the relative mobility of a compound in the roots of different species. It is not clear which properties determine the retention of a compound in the root cells, controlling the amounts translocated to the foliage.

The experiments in this section were designed to examine the

extent to which uptake could account for selectivity between *B. sterilis*, *B. willdenowii* and barley. Investigations were carried out to determine:-

i) the contribution of the sub-surface shoot to uptake of isoproturon from soil;

ii) the effects of seed depth on phytotoxicity;

iii) the effects of isoproturon on fresh weights and chlorophyll contents of liquid culture-grown plants;

iv) ^{14}C-isoproturon uptake rates into root and shoot tissue of the three species.

5.2 MATERIALS AND METHODS

5.2.1 Localised uptake from soil

This experiment was designed to separate root and shoot sections of seedlings so as to assess their relative contribution to uptake (Eshel and Prendeville, 1967). Pre-germinated 1 cm seedlings were planted in double plastic pots, such that their seed and root systems were exposed to soil in the lower pot, and their shoots to the soil in the upper (Figure 5.1 and Plate 5.2). The lower pot (9 cm high) was filled with 200 g of air-dried soil to within 4 cm of the top.

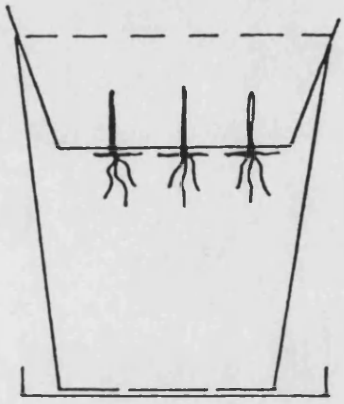


FIGURE 5.1
The double pot technique for studying root *versus* shoot uptake.

The upper pot (4.5 cm high), of the same diameter, was fitted tightly into the lower pot. The base of the upper pot had six holes of 5 mm diameter, through which the seedlings protruded, attached by a ring of non-toxic pliable putty. The lower pot was watered to field capacity before sealing the holes with putty. The upper pot was then filled with 125 g of air-dried soil and watered.

Isoproturon at 2 kg a.i.ha^{-1} was applied as a drench prior to sealing, to the soil in the lower pot, upper pot or both compartments. Control pots were supplied with distilled water. Overhead and sub-irrigation were carried out every second day to maintain the soil at field capacity. Fresh weights of the shoots were measured 21 days after planting and expressed as a % of the control.

5.2.2 Seed depth effects

Pre-germinated seeds were sown at 0.5, 2 and 4 cm depth in John Innes No. 2 compost, maintained throughout the experiment at 75% pot capacity. Isoproturon was applied at sowing in 10 cm^3 of distilled water to each pot by pipette, spreading evenly over the surface the equivalent of 2.5 kg a.i.ha^{-1}. Control pots were given 10 cm^3 of distilled water. After 21 days, fresh weights of shoots were recorded and the results expressed as a % of control plants.

5.2.3 Uptake from nutrient culture

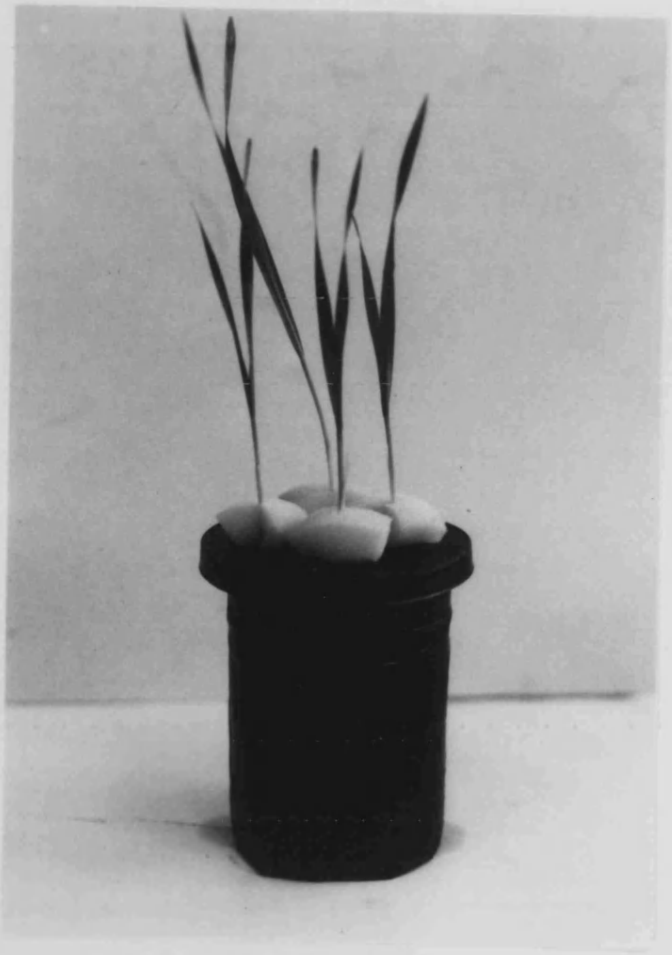
Pre-germinated 2 cm seedlings were individually transferred from seed trays into blackened liquid culture beakers of 400 cm^3. The seedlings were supported just above seed level by foam-lined lids which held four plants each (Plate 5.1). Each beaker

PLATE 5.1

Plants grown in the liquid culture system.

PLATE 5.2

The double pot technique in practice.



contained 350 cm^3 of full strength Long Ashton solution (Hewitt, 1966) (see Appendix 11.1), which was in contact with the seedlings from the level of the seed downwards. Aeration was maintained by leaving a space between the solution and the lid. The solution was renewed every second day.

Plants were grown for 14 days in liquid culture, to which isoproturon was then added at concentrations of 0.001, 0.01, 0.1 and 1.0 mM. After 24 hours, the roots were rinsed in distilled water and nutrient solutions replaced.

Fresh weights of shoots were measured 7 days after application of the herbicide. The chlorophyll content of these shoots was determined for each concentration of isoproturon. A sample of 50 mg of leaf tissue from similar positions on leaf 1 was suspended in 5 cm^3 of 80% acetone and maintained at 0°C in the dark for 5 days, at which time all the chlorophyll had leached out of the leaf into solution. Samples were determined at 645 and 663 nm, using a 'Shimadzu UV-260' recording spectrophotometer to obtain the levels of total chlorophyll, as reported in the method of Arnon (1949).

5.2.4 Radioisotope determinations

5.2.4.1 Source of activity

A supply of ^{14}C-isoproturon, labelled on the isopropyl group, with a specific activity of 0.76 MBq mg^{-1} (21 μCi mg^{-1}) and a radiochemical purity of 98%, was kindly donated by Ciba-Geigy, U.K. A stock solution of ^{14}C-isoproturon was prepared by dissolving the solid in acetone:water (1:2 v/v). An aliquot of this solution was added to each vessel containing Long Ashton nutrient solution to give a 1 μM concentration (0.17 KBq cm^{-3}; 4.6 x 10^{-3}

$\mu Ci\ cm^{-3}$) (This level was insufficient to cause toxic effects in the plant).

5.2.4.2 Plant material

Plants were grown as before, in blackened beakers containing 350 cm^3 of nutrient solution, and at 14 days old, transferred to individual glass vessels of 4 cm height (Plate 5.3). Each vessel contained 7 cm^3 of nutrient solution and a 1 μM concentration of ^{14}C-isoproturon. The plants were held in position, with the roots submerged in solution, using a circular foam disc resting on the rim of the vessel. During the experiment, plants were maintained in a 'Conviron S10h' growth cabinet in the conditions stated previously.

5.2.4.3 Solution sampling procedure

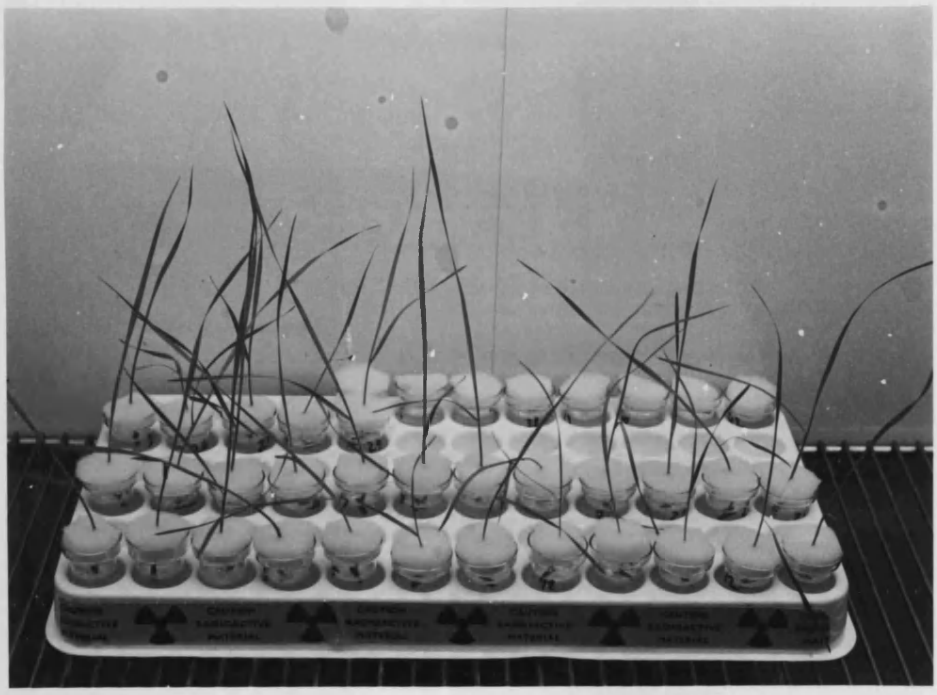
At commencement, and at two hourly intervals after the addition of ^{14}C-isoproturon, 250 $\mu\ell$ samples of the solution around the roots were removed from eight individual vessels using a 250 $\mu\ell$ 'Hamilton' microsyringe, which penetrated the foam discs. Each sample was transferred to a clean 'Packard' polyethylene liquid scintillation vial, to which 5 cm^3 of 'LKB Optiphase safe' scintillant was added. Each vial was shaken for 30 sec and then left to stand for two hours before counting, to avoid chemiluminescence.

5.2.4.4 Tissue sampling procedure

At each assessment period, three plants were removed and excised at seed level. Roots were rinsed in distilled water for 2 min, blotted dry and weighed, and shoots were excised

PLATE 5.3

The vessels used for root applications of ^{14}C-isoproturon in section 5.2.4.2.



just above the seed and weighed. Separately, shoot and root sections were cut into small strips of 0.5 cm and placed into scintillation vials. 0.5 cm^3 of 'Packard Soluene-350' tissue solubiliser was added to each vial, prior to placing them in an oven at 60°C for 15 hours.

Because of the excessive colour quenching caused by chlorophyll, extracts were decolourised before counting. After cooling, the vials were agitated for 30 sec, then 0.5 cm^3 of chlorine water (6.5 g of Cl_2 per litre of water) added to each shoot sample. Decolourisation occurred within five seconds. Finally, 5 cm^3 of 'Optiphase' scintillant was added to the extracts and each vial shaken for 30 sec before being left to stand for 2 hours.

5.2.4.5 Counting technique

All vials containing solution and plant extracts were counted using an 'LKB Wallac 1217 Rackbeta' liquid scintillation counter. The count rate for each sample of aqueous solution was compared with that of a standard prepared in exactly the same manner, but without a plant present. Count rates for all vials were corrected to allow for the level of background radiation. A control vial, containing no extracted or radioactive material, was always included in every batch of vials counted. Aqueous samples contained Long Ashton solution as a control, and tissue samples, an untreated plant.

5.2.4.6 Quench correction

The counting efficiency of a system containing 'Soluene-350', chlorine water and 'Optiphase', but no plant material was regarded as 100% throughout this work. However, 'Soluene' and

chlorine water caused marked chemical quenching, and the true counting efficiency of this system was nearer to 75%. Colour quenching by chlorophyll was avoided by using a decolouriser.

5.2.4.7 Evaporation correction

At each assessment period, the quantity of liquid in three vessels, both with and without a plant present, was measured to adjust for evaporation and transpiration. The amount remaining at each period was calculated and the disintegrations per minute of each aqueous sample adjusted accordingly i.e.

$$\frac{\text{dpm}}{0.25\text{cm}^3} \times (7\ \text{cm}^3 - \text{quantity lost})$$

5.2.4.8 Weight correction

Adjustments were also made to correct for differences in plant size. Count rates of each sample were divided by the fresh weight of the tissue, and expressed as dpm per 100 mg of plant tissue.

5.3 RESULTS

5.3.1 Phytotoxicity following exposure of the sub-surface shoot and roots

The toxicity of isoproturon applied to the sub-surface shoots of plants growing in soil was compared with its toxicity when applied to all subterranean parts. Figure 5.2 shows the growth reduction of treated plants, expressed as a % of untreated plants, 21 days after isoproturon application to the soil. Isoproturon proved to be more phytotoxic to all three species when present in the root rather than the shoot environs. Restriction of isoproturon to the region above the seed resulted in negligible decreases in fresh weight, being on average 94.1% of the control. Greater reductions in fresh weights resulted from root and root/shoot applications, growth being 44% and 40% of the control respectively. The three species showed a similar response to a specific treatment, but site of application produced a significantly different response ($P = 0.001$).

5.3.2 The effect of seed depth on phytotoxicity following soil surface treatments

Figure 5.3 shows the effect of isoproturon in the topsoil on subsequent growth following seedling emergence from various depths. The greatest reductions in growth resulted from seeds sown at 0.5 cm for *B. sterilis* (17% of control) and 2 cm for *B. willdenowii* and barley (79% and 91% respectively). Effects of isoproturon on seeds at 4 cm depth were negligible for all species, decreasing growth by 4% on average. Species did react differently to sowing at shallower depths ($P = 0.001$), yet the correlation between depth and phytotoxicity was not straightforward.

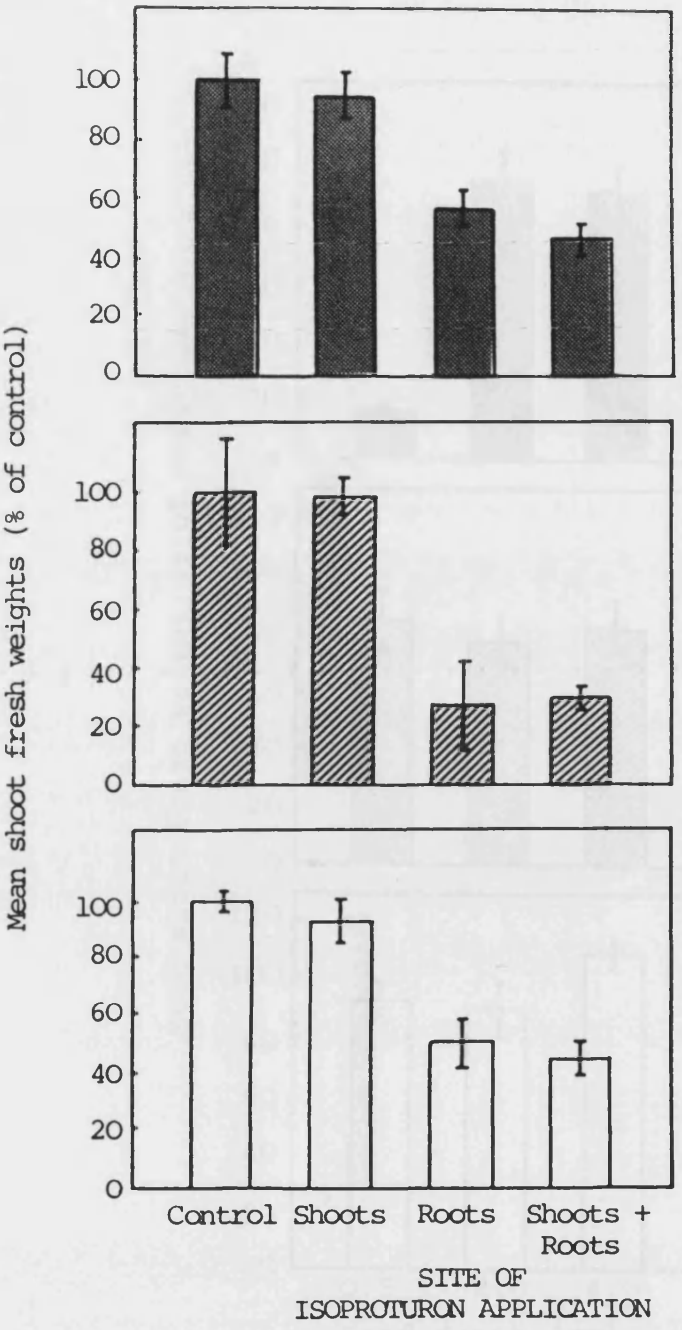


FIGURE 5.2

Shoot fresh weights following 21 days growth in soil containing 2 kg a.i. ha^{-1} isoproturon in shoot or rooting zones.

B. sterilis , *B. willdenowii* and barley .

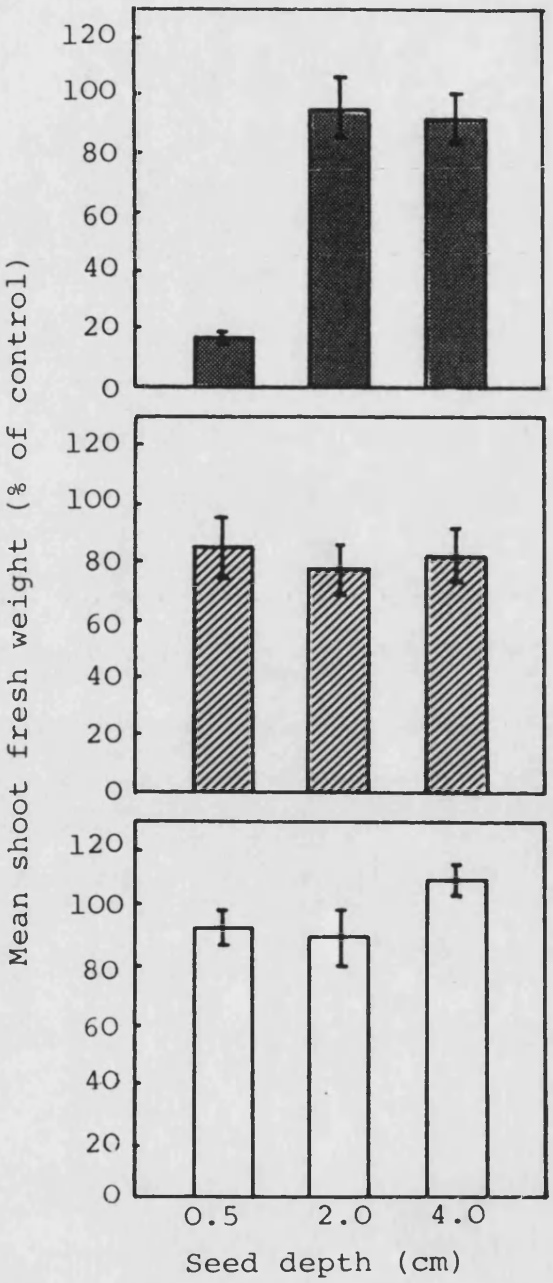


FIGURE 5.3

Growth reductions following 21 days growth in soil at 0.5, 2 and 4 cm depth, surface treated with 2.5 kg a.i. ha^{-1} isoproturon at sowing. *B. sterilis* ▓, *B. willdenowii* ▨ and barley □.

5.3.3 Phytotoxicity following exposure to isoproturon in nutrient culture

Figures 5.4 and 5.5 indicate the resulting changes in fresh weights and chlorophyll contents respectively of plants treated with isoproturon via the roots. Shoot fresh weights decreased as the concentration of isoproturon in the rooting medium increased. The decline was roughly linear for *B. willdenowii* and barley, but *B. sterilis* exhibited increased growth (over controls) at concentrations of 0.001 and 0.01 mM.

Chlorophyll contents did not correlate well with effects on plant growth, though they decreased linearly with respect to concentration. Low concentrations of isoproturon produced increased chlorophyll contents in barley and *B. willdenowii*. *B. sterilis* lost pigment at every concentration tested, though in smaller quantities than barley at the highest doses. Species were significantly different at $P = 0.001$.

5.3.4 The uptake of ^{14}C-isoproturon by whole plants

Figure 5.6 shows the loss of ^{14}C-isoproturon from the nutrient solution. Plant roots submerged in nutrient solution containing ^{14}C-isoproturon absorbed between 53 and 77% of the applied dose from the solution over the 7 day period. The greatest losses occurred from solution containing barley plants, the rate of uptake being linear in this species. Uptake into *B. sterilis* seedlings followed a similar pattern, but at a slower rate, total absorption not exceeding 53% of the applied dose. Rates of absorption by *B. willdenowii* were initially most rapid, but slowed down considerably after 2 days.

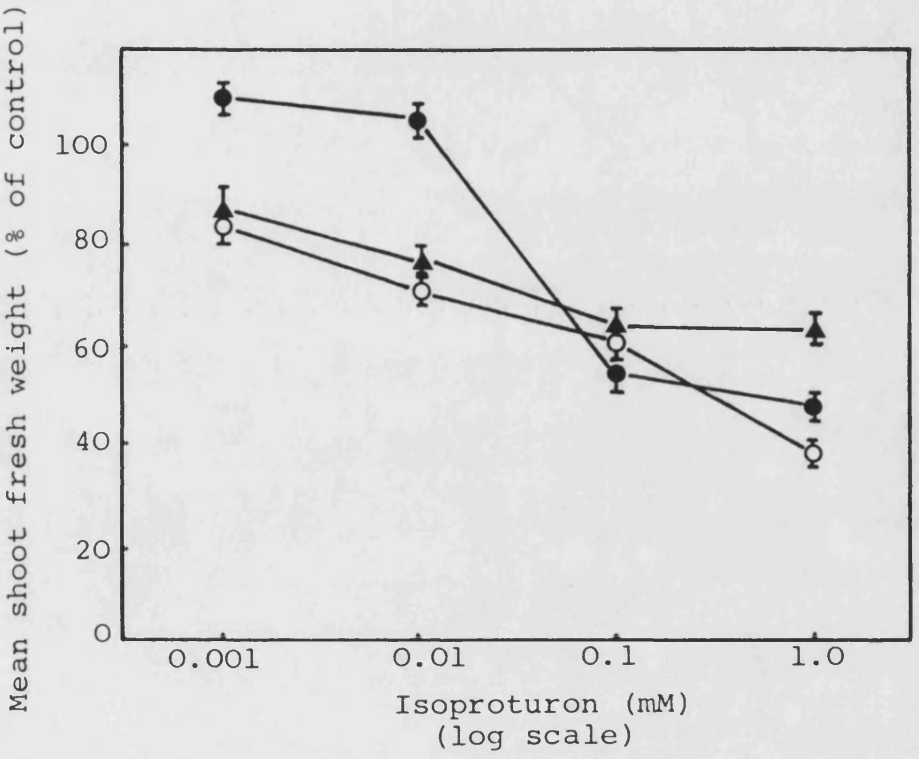


FIGURE 5.4

Shoot fresh weights following 7 days growth in nutrient culture treated with a range of isoproturon concentrations for 24 hours on day 1.

B. sterilis (●), *B. willdenowii* (O) and barley (▲).

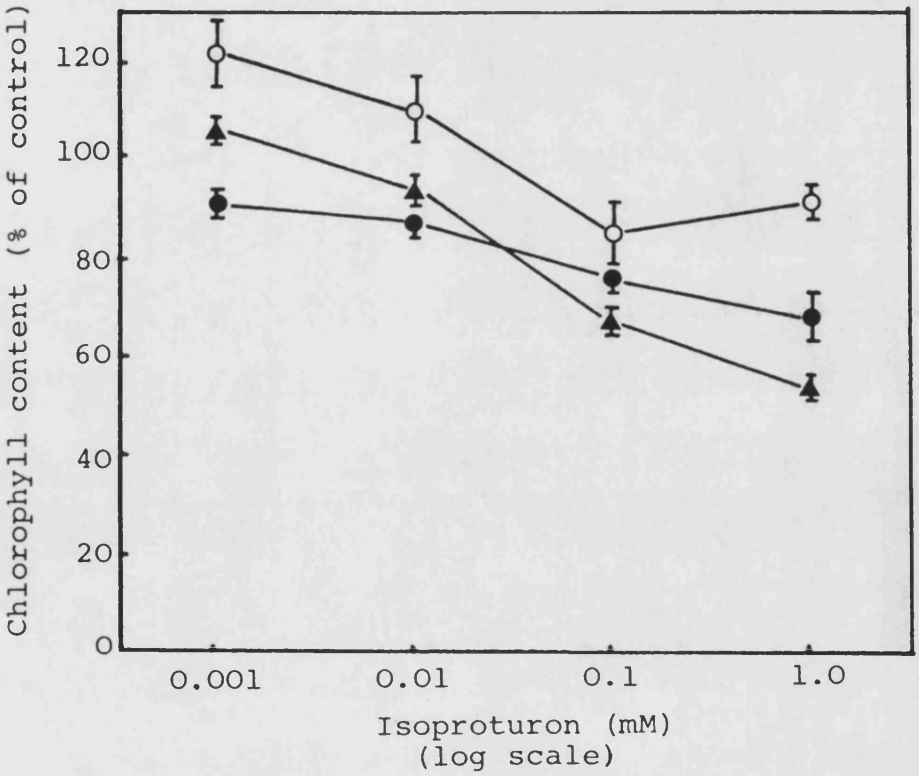


FIGURE 5.5

Chlorophyll contents following 7 days growth in nutrient culture treated with a range of isoproturon concentrations for 24 hours on day 1.

B. sterilis (●), *B. willdenowii* (○) and barley (▲).

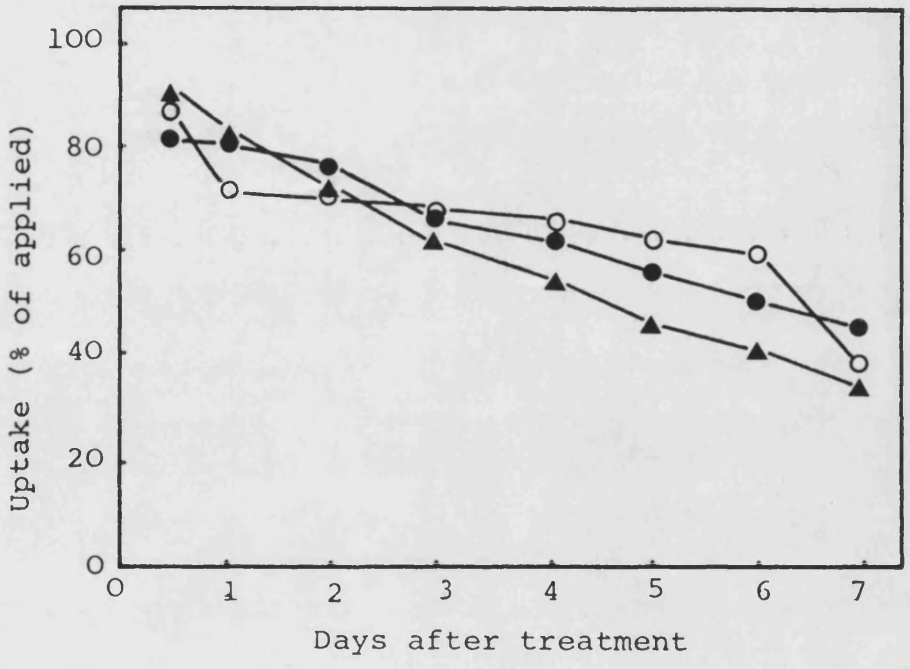


FIGURE 5.6

Uptake of ^{14}C-isoproturon following its application to nutrient solution containing roots of *B. sterilis* (●), *B. willdenowii* (○) and barley (▲).

Figures 5.7 and 5.8 show rates of uptake of ^{14}C-isoproturon into roots and shoots respectively, of the three species. All the ^{14}C present in each sample was assumed to be still contained in isoproturon molecules. Uptake into shoots and roots followed a similar pattern, although it varied between species. Barley roots took up much smaller total amounts of ^{14}C-isoproturon, and at a slower rate than the *Bromus* spp. A relatively small percentage of ^{14}C-isoproturon was found in the shoots, even after 7 days. *B. sterilis* seedlings absorbed the largest amounts over the first 24 - 48 hours, and a large proportion of this ^{14}C-isoproturon moved into the shoots, maintaining a steady increase in uptake into leaf tissue over the 7 day period. Rapid uptake into *B willdenowii* followed an initial lag phase, this species absorbing the greatest quantities overall.

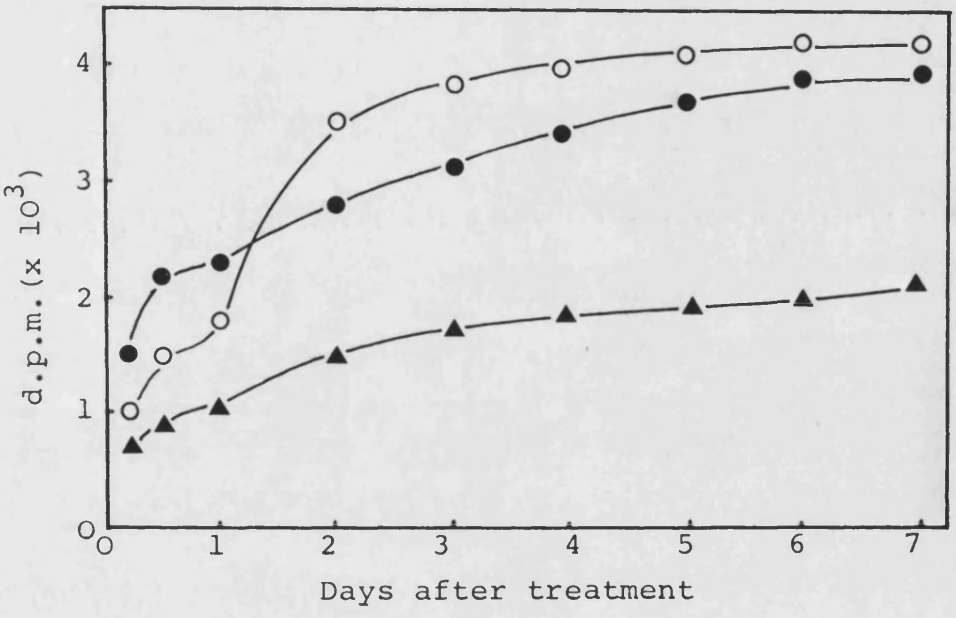


FIGURE 5.7

Disintegrations per minute (per 100 mg) of root tissue following application of ^{14}C-isoproturon to nutrient solution containing roots of *B. sterilis* (●), *B. willdenowii* (O) and barley (▲).

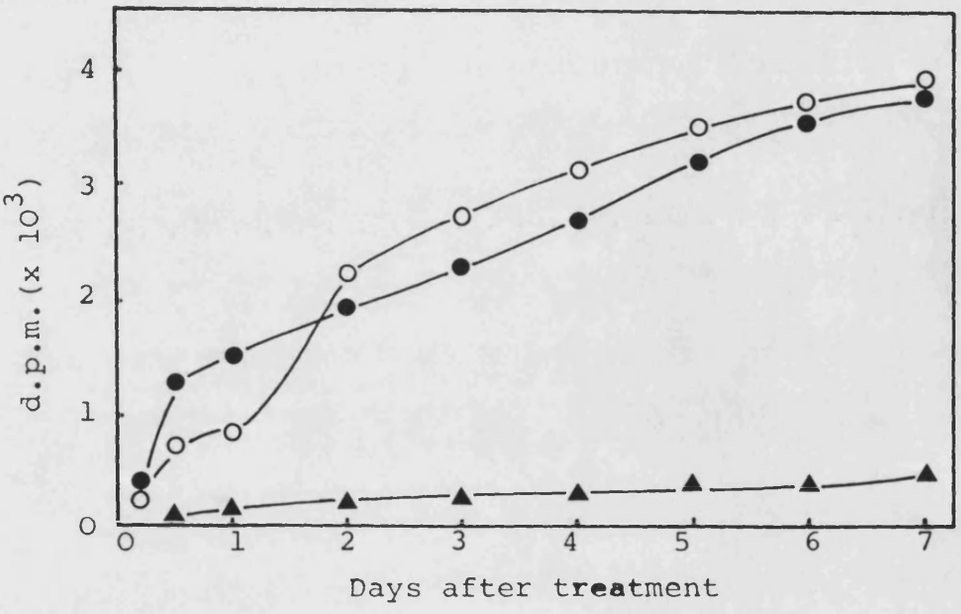


FIGURE 5.8

Disintegrations per minute (per 100mg) of shoot tissue following application of ^{14}C-isoproturon to nutrient solution containing roots of *B. sterilis* (●), *B. willdenowii* (O) and barley (▲).

5.4 DISCUSSION

i) The method of Eshel and Prendeville (1967) was chosen to investigate the site of uptake from soil, since other techniques, such as the use of activated charcoal, may alter availability of the herbicide by increased adsorption. (Priest, 1976). The present results agree with those of Nishimoto *et al.* (1967) and Prendeville *et al.* (1967), that photosynthetic inhibitors are more effective when placed in the root zone than in the shoot zone of plants, although injury was obtained from shoot zone exposure. Only in a couple of cases has shoot zone uptake alone accounted for plant damage (Parker, 1966; Knake *et al.*, 1967). This is likely to be a result of direct access of the herbicide to a vital organ such as the coleoptile node in grasses (Rahman and Ashford, 1970).

The subterranean shoot may be vulnerable while the seedling is emerging, but once at the surface, it grows solely by lateral expansion, exposing only limited new areas. The shoot also tends to be rather unwettable, so dissolution and diffusion of the herbicide cannot be assisted by water uptake.

The speed with which the sensitive region of a plant reaches the soil and the length of time it remains there while still sensitive is important in determining susceptibility. In these three monocotyledonous species, work with diallate showed that the sensitive region is the growing point or apical meristem within the coleoptile sheath, which at the early seedling stages grows rapidly. Maximum effectiveness occurred if herbicide placement was such that the coleoptile was in contact with the treated soil continuously from initiation of its growth until it reached the soil surface (Parker, 1963). Parker (1963) observed that

the mesocotyl of wild oat plants forced the coleoptile node upwards into the surface layer of treated soil early in its development, whereas in barley this sensitive site remained close to the seed initially.

ii) Blair (1978) proposed that the development of the seedling roots in relation to the position of the herbicide in the soil was the most important criterion for plant damage. Isoproturon was more damaging to wheat, wild oat and blackgrass when incorporated below compared with above the seed in soil, however, Blair (1978) used the charcoal layer technique. More recent work has shown that his conclusions still hold when activated charcoal is substituted by plastic straws which had no independent action (Addala *et al.*, 1985). Seed depth in soil has been the cause of differential uptake between species, as indicated by fresh weight loss. The smaller size of *B. sterilis* seedlings may account for the enhanced damage resulting from sowing near the soil surface within the zone of isoproturon activity i.e. 0.5 cm. The roots of pre-germinated seeds may act like adventitious roots, since it is difficult to ensure that their depth upon transfer to soil simulates the normal pattern of distribution. Although the same dilemma occurs with barley and *B. willdenowii* roots, their fast growth rate may allow them to reach beyond the zone of herbicide activity before damage ensues. Ayres and Richardson (1981), however, suggested that *B. sterilis* was relatively tolerant to soil-acting herbicides in pot trials, since the roots and shoots moved through treated soil very quickly.

The above discussion is based on the assumption that isoproturon remained localised in the top 1 cm layer of soil.

However, Siriwardana *et al.* (1981) recorded isoproturon movement down the profile to a depth of 4 - 5 cm in 30 days, with the daily addition of 10 cm^3 of water to the surface. Nevertheless, Addala *et al.* (1985) suggested that for shallow germinating species with herbicides of similar physical and phytotoxic properties to chlortoluron, the solvent action of rainfall, together with diffusion, is enough to allow the transport of toxic quantities to the target plant, although any leaching action is likely to increase activity. The extent of downward movement of isoproturon applied to the soil surface would seem to be a critical factor in the selectivity between barley and *B. sterilis* seedlings due to the marked difference in root distribution. At a comparable physiological age, barley roots are larger and may be able to absorb water from areas beyond the leaching capabilities of the herbicide over a given period. This would result in dilution of the herbicide within the tissue, thus causing less damage to barley plants. From this experiment However, it is difficult to conclude that barley and *B.willdenowii* plants absorb less isoproturon from the soil, because possible reasons accounting for their reduced damage cannot be separated into individual factors. Tolerance may result from reduced absorption, penetration and/or movement within the plant to the site of action, and possibly increased detoxification.

iii) Differences between species relating solely to uptake can be investigated using a radio-labelled herbicide by quantifying the amounts absorbed from liquid culture. Applying herbicides in this manner ensures their availability to the root surface, and eliminates such variables as leaching. However, experiments carried

out in nutrient culture are not comparable to those using soil, where additional external physical mechanisms can operate. In fact, differential absorption in two plant species can be the reverse in nutrient solution when compared with soil (Geissbühler *et al.*, 1963a).

Meaningful conversion of kg a.i.ha^{-1} to a molar concentration is impossible, but experiment 5.3.3 served to quantify the effects of isoproturon at a range of concentrations upon fresh weights and chlorophyll contents. Losses in fresh weight suggest impaired photosynthetic activity, leading to growth retardations and subsequent death. However, isoproturon was removed from the root environs after 24 hours, giving the seedlings six days to recover. Reductions in fresh weight from high concentrations were smaller in barley than in *Bromus* plants, indicating that the former species can more easily resume its normal growth rate after herbicide treatment. Low concentration of isoproturon promoted the growth of *B. sterilis* seedlings, similar findings being reported by Fedtke (1973) for effects of low doses of the urea, methabenzthiazuron on wheat plants. His work proposed that, following herbicide breakdown, the photosynthetic and metabolic capacity of the plants was increased, due to changes in the amino acid, water soluble protein and pigment ratios. However, the present results are unlikely to represent 'physiological effects' induced by isoproturon, since the time scale of the experiment was considerably shorter than that of Fedtke (1973).

Chlorophyll synthesis generally occurs rapidly during the first seven days after germination once the leaf is illuminated. The chlorophyll content per unit fresh weight or leaf area increases

markedly during leaf development, reaching a maximum before leaf expansion is complete. In grasses, the chlorophyll content per unit leaf area does not decrease until about 20 days after completion of expansion (Friend, 1961)., Loss of plant pigmentation is often the first visible symptom following application of a photosynthetic inhibitor herbicide. Chlorophyll loss correlated well with membrane breakdown in flax cotyledons treated with monuron (Pallett and Dodge, 1980), thus it is a good indicator of plant sensitivity to a herbicide. Clark (1984) recorded reduced chlorophyll contents in sensitive purple nutsedge *(Cyperus rotundus* L.) but not in tolerant maize plants treated with M & B 34552, and related this to an inhibition of chlorophyll synthesis. Chlorophyll contents of all three species in this study decreased linearly with increasing concentrations of isoproturon. Chlorophyll levels in barley leaves treated with high concentrations were surprisingly lower than those in *Bromus* leaves, when expressed as a percentage of the control, suggesting that damage to the photosynthetic apparatus was more severe in the former species. *B. willdenowii* showed enhanced chlorophyll levels at low doses, implying either that synthesis was promoted or breakdown was delayed. Fedtke (1973) recorded delayed chlorophyll degradation in wheat plants treated with low doses of methabenzthiazuron, although the effect was not obvious until twelve days after treatment. The stimulatory effect of sub-toxic levels of herbicides implies that the plant has fully detoxified the compound. An attempt to elucidate the implications of this will be made in a later section of this thesis.

iv) By quantifying the effect of isoproturon at a range of concen-

trations upon fresh weights, it was established that a concentration of 0.001 mM was non-phytotoxic to the species concerned in this study. It is assumed that uptake is constant for a given concentration, though the rate may be concentration-dependent. The rate of uptake of ^{14}C-isoproturon by the three species was not significantly different, as indicated by loss of the labelled herbicide from the nutrient solution. The suggestion that barley plants absorbed slightly greater amounts may be due to their larger root surface. In the following experiment, where plant size was taken into account, barley root tissue absorbed relatively low levels of ^{14}C-isoproturon throughout the 7 day period. This suggests that isoproturon has more difficulty in penetrating the root epidermis of this species. However, it is doubtful whether any cuticle covers the young extending roots (Martin and Juniper, 1970), thus the first barrier to the passage of molecules must be the Casparian strip. Poor membrane permeability in barley roots may perhaps partially account for the slow accumulation of isoproturon. It is possible that the root surface of barley possessed fewer root hairs than the *Bromus* spp., and since these hairs greatly increase the surface area available for absorption, their absence would suggest reduced uptake. However, there is no evidence for the infrequency of root hairs in this species.

There was a rapid uptake of isoproturon into *B. sterilis* over the first 24 hours, subsequent accumulation remaining at a steady rate. Despite the initially slower uptake into *B. willdenowii* tissue, penetration into this species must have been equally as rapid as in *B. sterilis* to account for the large quantities measured in root tissue. It can only be assumed however, that all the

^{14}C-isoproturon bound on to the root surfaces was washed off adequately. The similarity in the pattern of uptake into root and shoot of a given species implied that movement from root to shoot tissue was not limiting uptake into the latter. However, xylem loading and transport rates may vary between species, influencing uptake into the shoot. Differential translocation will be investigated in the following section.

Therefore, if uptake during the first week after herbicide application is indicative of subsequent rates of absorption from the root solution, this factor may be important in species selectivity to isoproturon. Nevertheless, there is a danger in extrapolating this result to the soil situation since other variables will influence uptake in the field.

6. HERBICIDE TRANSLOCATION

6.1 INTRODUCTION

Selectivity between species may result from differential translocation of a herbicide following uptake (Robertson and Kirkwood, 1970). It is now generally accepted that after a chemical has penetrated the leaf tissue it has three possible routes of distribution; it can move within the apoplast, the symplast, or remain immobilized in the tissue at the point of entry (Price, 1973). The apoplast is an interconnected system throughout the plant consisting of the non-living cell walls and xylem vessels, and long-distance movement of solutes is a passive one within the transpiration stream, for the most part in the xylem. Phloem translocated herbicides appear to follow the same route as assimilates through the sieve tube system, and although the mechanisms involved remain only partly understood, there is evidence to suggest that movement occurs from 'source' (region of sugar synthesis) to 'sink' (region of sugar demand). Some chemical compounds are apparently unable to penetrate the symplast and move only in the apoplast, although all herbicides must eventually enter the symplast to exert their action on the living processes of the plant. Crafts (1959) proposed that the substituted ureas moved primarily in the apoplast, although symplastic movement has since been identified (Emami Saravi, 1979). It is possible that extensive movement could occur within susceptible species while the herbicide is immobilized in the resistant plant (Kirkwood *et al.*, 1972). For example, poor translocation of the urea herbicide chlorbromuron in coriander *(Coriandrum sativum* L.) conferred resistance to this species (Hogue, 1978).

More commonly, the rate of translocation is the cause of variations in susceptibility between species to a particular herbicide (Geissbühler *et al.*, 1963a). Movement may be affected by temperature (Richardson, 1975), humidity (Veerasekaran *et al.*, 1976), light intensity (Leonard *et al.*, 1968) and soil moisture stress (Wills and Basler, 1971), yet rates have been seen to vary even under the same environmental conditions (Rogers and Funderburk, 1967). Bingham and Shaver (1971) found variations in the rate of diphenamid transport in tomato *(Lycopersicon esculentum* Mill.), bermudagrass *(Cynodon dactylon* L.) and winged euonymus *(Euonymus alatus* (Thunb.) Seib. Abei and Ebner (1962) recorded more rapid absorption and translocation of the urea chloroxuron in gallant soldier *(Galinsoga parviflora* Cav.) than in black bindweed.

Until the introduction of radioisotope labelling of herbicides (Yamaguchi and Crafts, 1958), methods for estimating the direction and rate of translocation were often crude. Though a qualitative rather than a quantitative assay, autoradiography is a useful method for ascertaining the direction of movement of radioisotopes following application to a particular region of the plant. Liquid scintillation counting allows the rate of herbicide translocation to be estimated.

Experiments were designed to investigate:-

i) the direction of movement of foliar-applied ^{14}C-isoproturon within *B. sterilis*, *B. willdenowii* and barley plants;

ii) the rate of accumulation of foliar-applied ^{14}C-isoproturon in leaf tissue distal to the application site.

6.2 MATERIALS AND METHODS

6.2.1 Application of ^{14}C-isoproturon

Plants were grown in liquid culture for 14 days prior to treatment. A stock solution of ^{14}C-isoproturon dissolved in acetone was applied as a 5 µl droplet using a 'Gilson' micropipette to the adaxial surface of the first leaf, 5 cm from the tip. The droplet spread out over a 0.5 cm^2 area, sectioned off from the rest of the leaf using two strips of masking tape (Plate 6.1). The area was previously treated with a 3 µl droplet of 0.2% 'Tween 20' wetting agent.

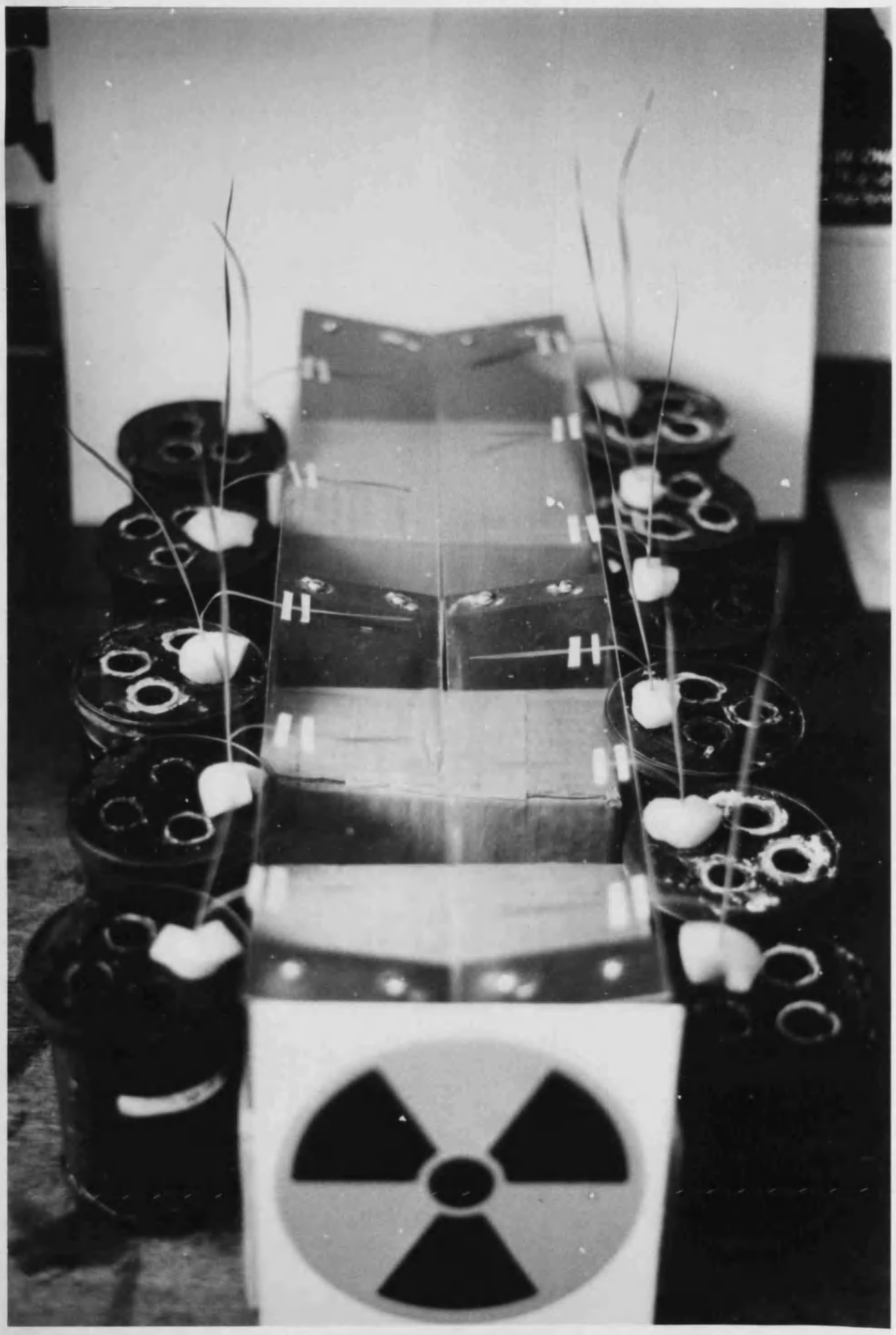
6.2.2 Autoradiography

At 0, 4 and 24 hours after treatment, the masking tape was carefully removed and the plant excised at seed level. Each shoot was placed on a heat-resistant acetate sheet and affixed with small strips of 'Sellotape'. The acetate sheet was sandwiched between two flat glass plates, and placed in an oven at 80°C for 2 hours to kill the tissue, and thus stop any further movement of the herbicide.

Each treated plant, still attached to the acetate sheet, was exposed to 'Kodak Industrex type CX2' X-ray film for 3 days. The film was subsequently developed for 3 - 5 minutes in 'Kodak D-19' X-ray developer and fixed for 5 minutes in 'Kodafix', using an intermediate stop bath of 1% acetic acid. Films were rinsed in running water for 15 minutes then air-dried.

PLATE 6.1

The application site of ^{14}C-isoproturon in section 6.2.1.



6.2.3 Translocation rates

At intervals after treatment, three 1 cm sections were excised from the treated leaf, 4, 3 and 2 cm above the application site (Figure 6.1, sections 1, 2 and 3 respectively). Each section was placed into an individual liquid scintillation vial and desolubilised in 0.5 cm^3 of 'Soluene-350' in an oven at 60°C for 15 hours.

After cooling and agitating, 0.5 cm^3 of chlorine water and 5 cm^3 of 'Optiphase' scintillant was added to each vial. Radioactivity was counted on a 'Rackbeta' liquid scintillation counter as described previously.

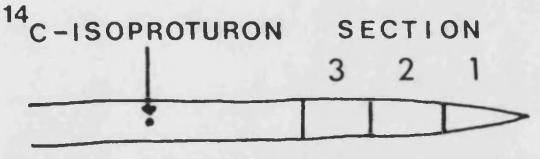


FIGURE 6.1
The site of application of ^{14}C-isoproturon in section 6.2.3.

6.3 RESULTS

6.3.1 Direction of movement of ^{14}C-isoproturon

Plant mounts and autoradiographs in Plates 6.2, 6.3 and 6.4 show the distribution of radioactivity in plants at different times following application of ^{14}C-isoproturon as a droplet to a single leaf of *B. sterilis*, *B. willdenowii* and barley respectively. The pattern of distribution of ^{14}C was similar in the three species. Acropetal movement of ^{14}C-isoproturon from the treated spot was detected four hours after application, and within 24 hours, ^{14}C had reached the tip of all three species. No detectable movement of ^{14}C occurred in the basipetal direction within the 24 hour period.

6.3.2 Rate of movement of ^{14}C-isoproturon

^{14}C-isoproturon applied as a droplet to the adaxial leaf surface of the first leaf moved at different rates towards the tip of the three species. Figure 6.2 shows the relative quantity of ^{14}C-isoproturon that accumulated in leaf tissue between 2 and 3 cm from the treated area (Figure 6.1, section 3). Rates of movement in *B. sterilis* leaves were substantially greater than in other species during the first 18 hours. High levels of ^{14}C-isoproturon were detectable in this species after 24 hours. Relatively low amounts accumulated in leaf tissue of *B. willdenowii* over this period, rates being slow and almost linear. Rates of movement into the leaf tip of barley plants increased with time, the greatest accumulation occurring between 18 and 24 hours after application. Movement into successive sections of the leaf followed a similar pattern (Figures 6.3 and 6.4). By 24 hours after treatment, similar levels of ^{14}C-isoproturon had accumulated in the leaf tissue of

PLATE 6.2

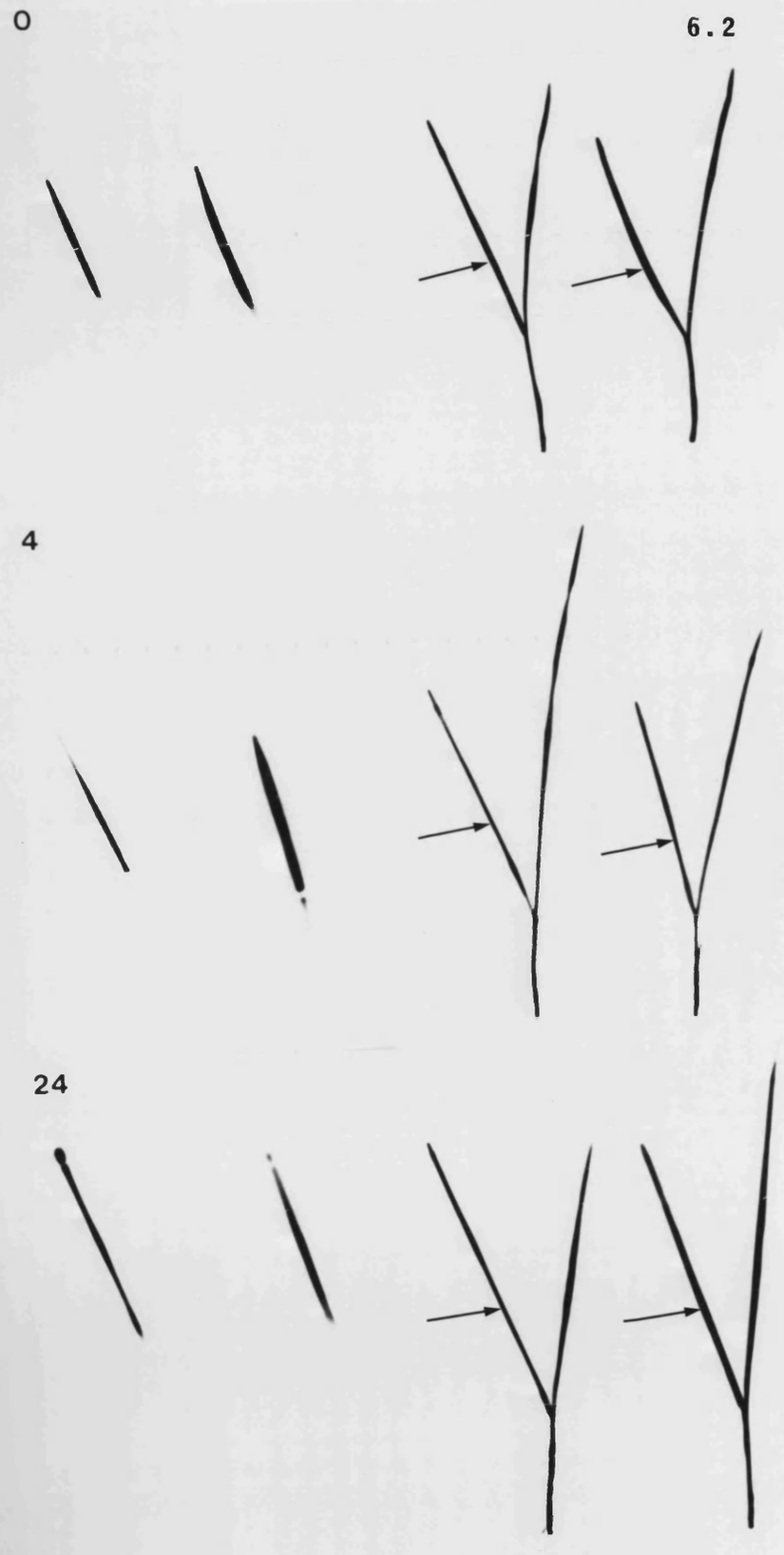
Autoradiographs (left) and plant mounts of *B. sterilis* at 0, 4 and 24 hours following applications of ^{14}C-isoproturon to the middle of leaf 1 (marked with arrow).

PLATE 6.3

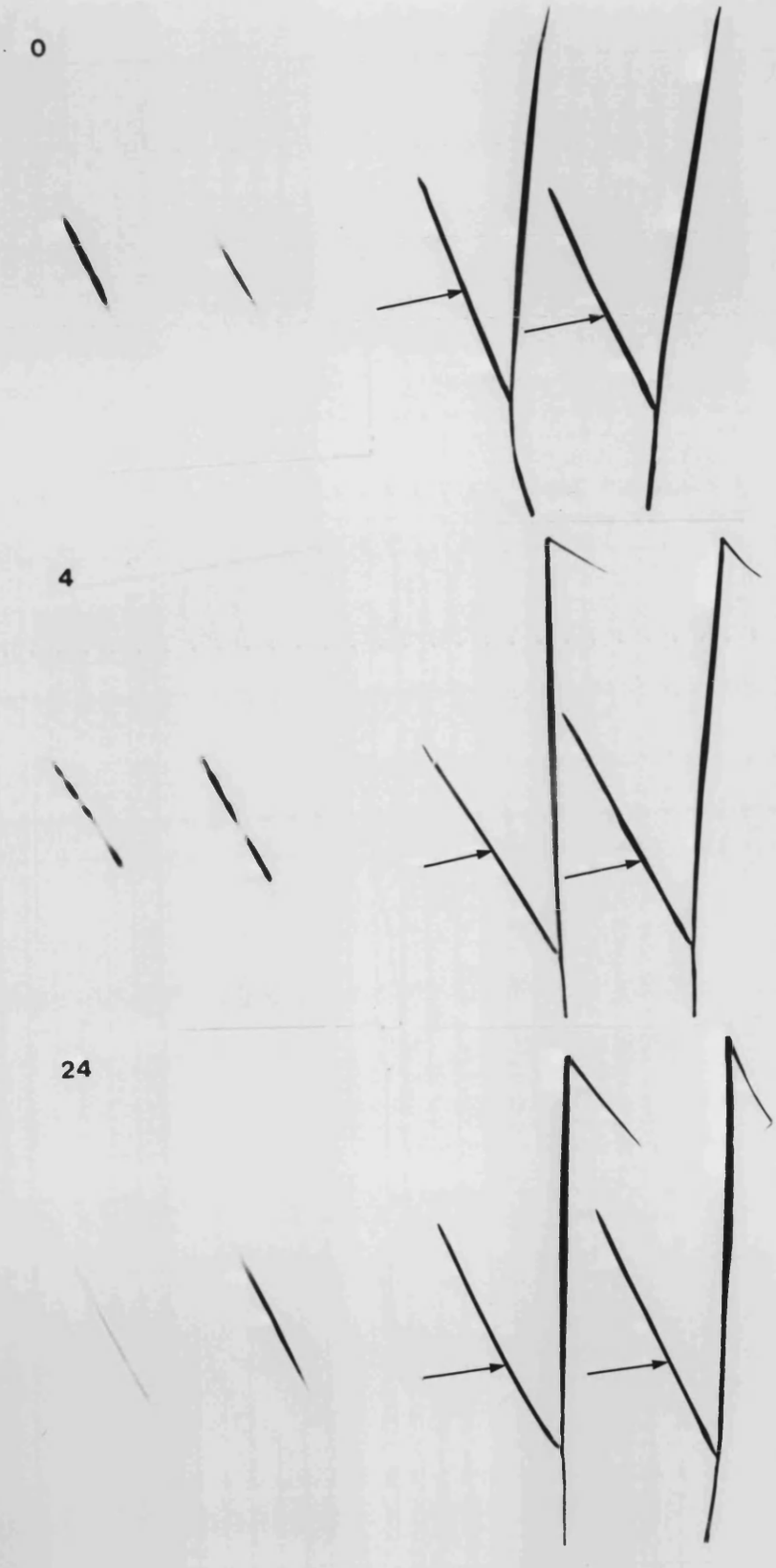
Autoradiographs (left) and plant mounts of *B. willdenowii* at 0, 4 and 24 hours following applications of ^{14}C-isoproturon to the middle of leaf 1 (marked with arrow).

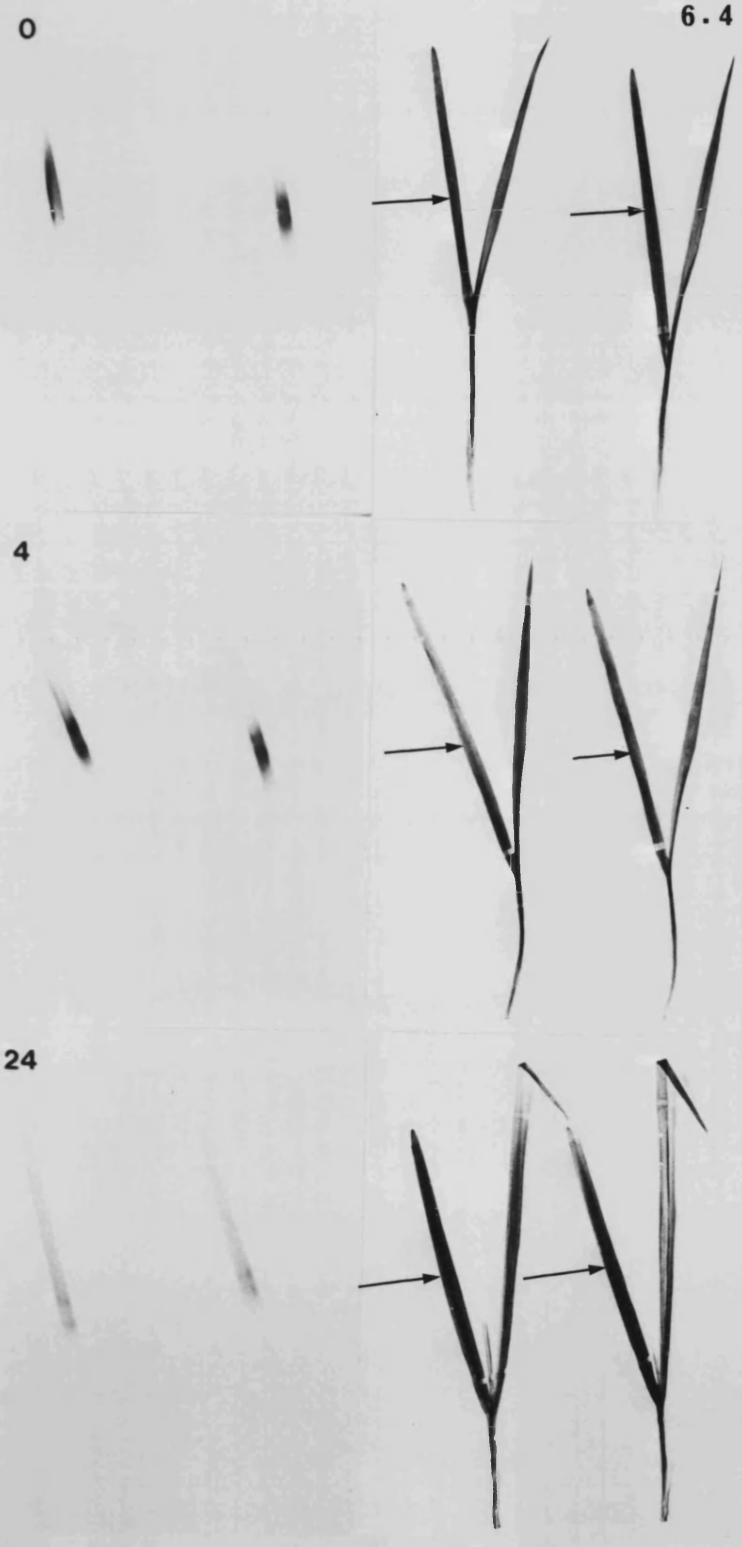
PLATE 6.4

Autoradiographs (left) and plant mounts of barley at 0, 4 and 24 hours following applications of ^{14}C-isoproturon to the middle of leaf 1 (marked with arrow).


0
6.2
4
24

6.3




0
6.4
4
24

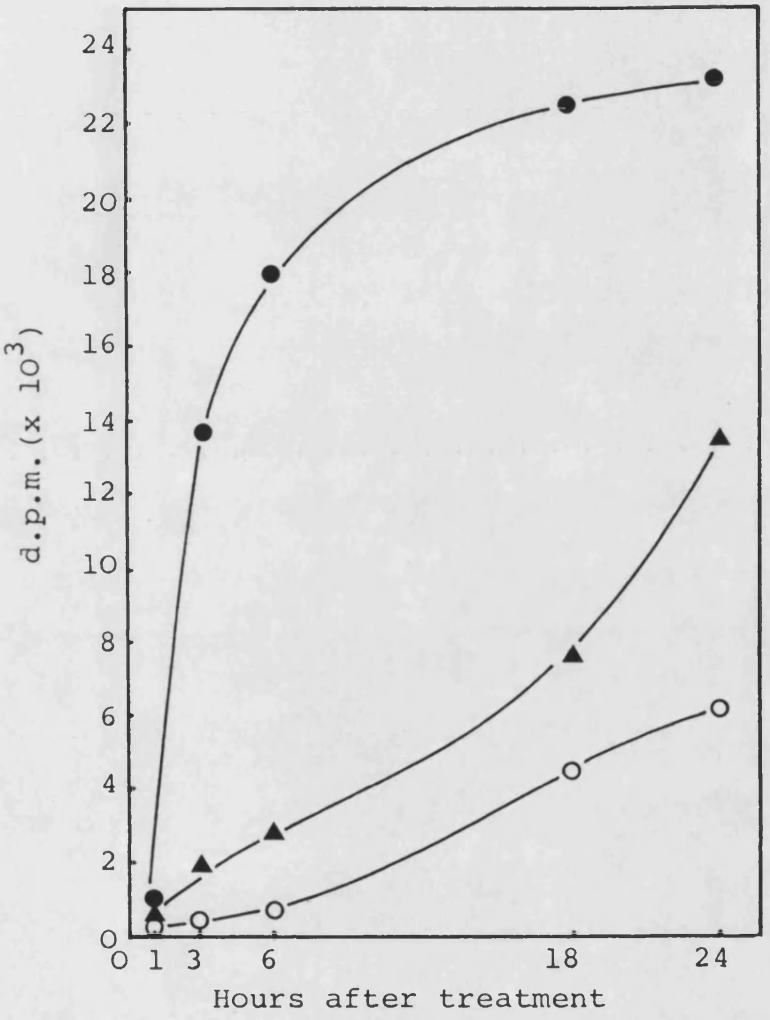


FIGURE 6.2

Disintegrations per minute of shoot tissue in section 3 (2-3 cm from the tip) following application of ^{14}C-isoproturon to the centre of leaf 1 (5 cm from the tip).

B. sterilis (●), *B. willdenowii* (O) and barley (▲).

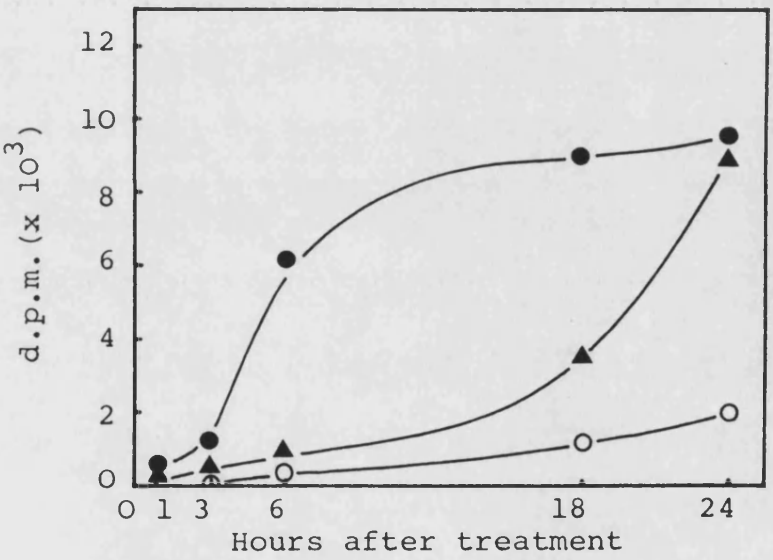


FIGURE 6.3

Disintegrations per minute of shoot tissue in section 2 (1-2 cm from the tip) following application of ^{14}C-isoproturon to the centre of leaf 1 (5 cm from the tip).

B. sterilis (●), *B. willdenowii* (O) and barley (▲).

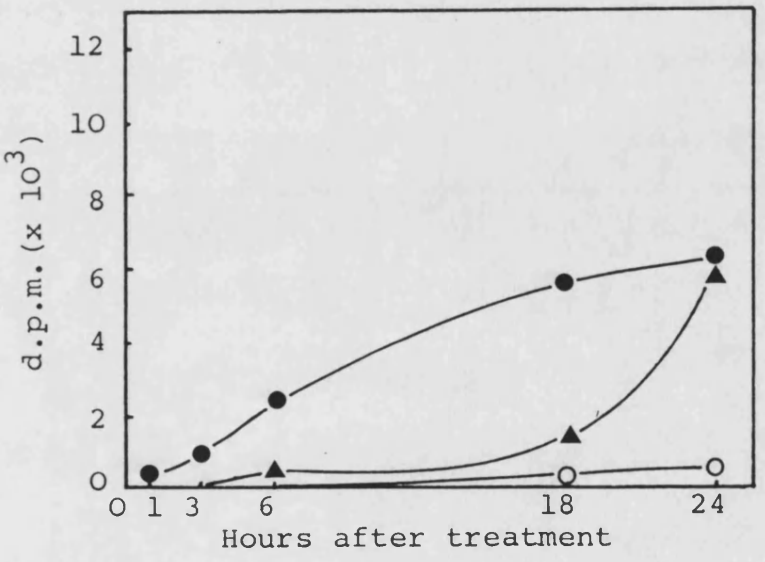


FIGURE 6.4

Disintegrations per minute of shoot tissue in section 1 (0-1 cm from the tip) following application of ^{14}C-isoproturon to the centre of leaf 1 (5 cm from the tip).

B. sterilis (●), *B. willdenowii* (○) and barley (▲).

B. sterilis and barley, and less than a quarter of this amount was present in *B. willdenowii*.

6.4 DISCUSSION

i) Experiments with radiolabelled compounds confirmed earlier ideas that the substituted ureas are primarily xylem-translocated herbicides (Muzik *et al.*, 1954; Crafts, 1959; Leonard *et al.*, 1966; Strang and Rogers, 1971). These compounds were concentrated in the cell walls and non-living vessels of the apoplast, and could not penetrate the membranes of living cells (Crafts, 1959). However, Emami Saravi (1979) claimed to have observed symplastic movement of ureas in wheat plants, and Nagi Reddy (1979) proposed that symplastic transport of isoproturon in blackgrass was partially responsible for the greater susceptibility of this species. Autoradiographs of the test species (Plates 6.2 - 6.4) indicated that there was little or no herbicide movement out of the treated leaf. Lack of detectable basipetal movement in *B. sterilis*, *B. willdenowii* and barley implied that ^{14}C-isoproturon was only transported in the xylem. Poor phloem translocation has also been observed for isoproturon in *B. sterilis* (Okereke *et al.*, 1981b) and in wheat, blackgrass and wild oat (McIntosh *et al.*, 1981). Discrepancies between this work and the earlier results of Nagi Reddy (1979) cannot be explained by differences in the age of treated leaf or the time scale of experimentation. Obviously all photosynthetic inhibitor herbicides have to enter the symplast to have their effect within the chloroplast, thus they must pass through the plasmalemma and protoplasm. Over small distances, movement can probably occur passively by simple diffusion.

Autoradiography is a useful tool for movement studies, but it does have limitations. It cannot be regarded as a quantitative technique, especially for compounds which undergo extensive

degradation by plants (Hay, 1976). It is important to equate the presence of the tracer with that of the herbicide. Careless preparation, for example, scratching or untimely exposure of film to radioisotopes, can cause artefacts to arise. Oven-drying of plant tissue has some advantages over freeze-drying. The former is faster to achieve and does not cause the material to become brittle, however, it may not have an immediate effect of stopping translocation. The use of glass plates to flatten tissue, and surfactants to ensure herbicide penetration, may cause the droplet to spread unnaturally. The hydrophilic/lipophilic balance of the surfactant is important in determining translocation (McIntosh *et al.*, 1981).

ii) By applying a droplet of surfactant prior to ^{14}C-isoproturon in experiment 6.2.1, any differences in the speed of penetration of the cuticle can be minimised. Therefore, rates of accumulation in the leaf tissue at the tip are considered to represent direct translocation rates. There were obvious differences in the amounts moving from the treated area to the tips of the three species. The rapid initial rate in *B. sterilis* suggests that xylem loading and subsequent movement was most efficient in this species. The delayed accumulation in barley implies that xylem loading was slower and, in *B. willdenowii*, both loading and movement inefficient. Börner (1965) found that linuron was rapidly translocated to the shoots of charlock (susceptible) following root application but in bean plants (resistant) movement was slow, accounting for the resistance of this species. Studies with the urea chlortoluron indicated greater rates of movement in wild oat than cereals (Ryan, 1981). Therefore, these

differences in translocation rates may partially account for the selectivity of isoproturon to *B. sterilis*, *B. willdenowii* and barley. Poor control of *B. willdenowii* in pot trials may in part be due to the slow translocation of isoproturon, thus preventing accumulation of the herbicide at metabolically-active sites.

7. HERBICIDE MODE OF ACTION

7.1 INTRODUCTION

It is well known that phenylureas exert their effect upon photosynthesis, inhibiting chloroplast electron transport on the reducing side of photosystem II. If electron flow is prevented, the reduction of NADP and the phosphorylation of ADP + ATP will cease. NADPH and ATP are required for the constant incorporation of carbon dioxide, thus an early manifestation of herbicide addition is the cessation of CO_2 uptake. The reduction of photosynthetic activity by inhibitors such as isoproturon can be measured on whole plants (van Oorschot, 1970), using carbon dioxide uptake as an indication of photosynthetic capacity. Recovery from inhibition after a short exposure of the roots to such herbicides has been used as a measure of the degree of their inactivation within the plant (van Oorschot, 1965; 1968).

Chlorophyll fluorescence was recognised quite early as a potentially powerful probe for the study of photosynthesis (Kautsky and Hirsch, 1931; McAlister and Myers, 1940). When green leaves are illuminated they fluoresce. Most of the fluorescence emanates from chlorophyll a in photosystem II (Papageorgiou, 1975) and constitutes that part of the excitation energy which cannot be dissipated through other channels, such as electron transport to NADP. An early step in electron transport is the reduction of the acceptor, Q_A, which, in its oxidised state accepts electrons, thereby quenching fluorescence (Duysens and Sweers, 1963). Reoxidation of Q_A makes an important contribution to the slow decline in the fluorescence peak which is rapidly established upon first illumination (Baker and Bradbury, 1981). Fluorescence yield following the instantaneous

increase upon illumination varies according to the ratio of $Q_{reduced}$ to $Q_{oxidised}$, and as such provides a direct measure of electron flow via photosystem II (Govindjee and Papageorgiou, 1971; Lavorel and Étienne, 1971). Herbicides which block electron flow between Q_A and plastoquinone can cause a marked increase in fluorescence by preventing the reoxidation of Q_A (Walker, 1981). It has been shown that the degree of increase in fluorescence as a result of photosynthetic inhibition is directly related to the concentration of the inhibitor (Zweig *et al.*, 1963), an observation which probably relates to the number of photosystem II reaction sites inhibited by a given herbicide concentration.

Pfister *et al.* (1979) suggested that the inhibitory activity of herbicides affecting photosystem II depends on their ability to bind specifically to the chloroplast membrane. The inhibitory potency of a herbicide in photosynthetic electron transport is expressed by the I_{50} value, the concentration required for 50% inhibition of chloroplast electron transport. The value can only be compared when the same electron donors or acceptors are used (Fedtke, 1982). The assay tests only limited sections of the electron transport chain, which has allowed the exact site of inhibitory action to be located (Trebst, 1980).

In this section, the effect of isoproturon on the site of action was investigated.

i) Photosynthetic rates were recorded in leaves of *B. sterilis*, *B. willdenowii* and barley following applications of isoproturon to the nutrient solution. By comparing the degree of inhibition in the three species, any differences in the amounts affecting the site of action could be identified. The

speed of recovery from inhibition was related to the efficiency of herbicide inactivation.

ii) Chlorophyll fluorescence was used as a tool to study the efficacy of isoproturon in electron transport inhibition. This allowed a different aspect of photosynthesis to be examined, for comparison with carbon dioxide uptake assays.

iii) The effectiveness of *in vitro* chloroplast electron transport inhibition by isoproturon was quantified using an oxygen electrode. The resulting I_{50} values were used to indicate susceptibility at the chloroplast level.

7.2 MATERIALS AND METHODS

7.2.1 Infra-red gas analysis

Plants of the three test species were grown in liquid culture for 14 days. Isoproturon was added to the root solution for 24 h at concentrations of 0.01, 0.1 and 0.5 mM. After 24 h, the roots in each beaker were rinsed in distilled water for 2 min and returned to fresh nutrient solution.

At intervals after treatment, a 0.1 g sample of tissue from the centre of leaf blades 1 and 2 (1st and 2nd leaves to emerge) was placed into the measurement chamber of an Infra-red 'series 225' gas analyser (Analytical Development Co. Ltd., Hoddesdon, U.K.). Flow rate was constant at 0.8 $\ell\ min^{-1}$ and the CO_2 content of the reference gas calibrated to measure photosynthesis in $\mu g\ CO_2\ g\ FW^{-1}h^{-1}$. Three replicate treatments were assayed at each period.

Formula:-

$$\frac{\text{deflection (units)}}{\text{calibration deflection (units)}} \times \text{ppm } CO_2 \text{ in reference air} \times CO_2 \text{ in sample air} \times \text{flow rate} \times \text{time (h)} \div g\ FW^{-1} \text{ of tissue}$$

7.2.2 Chlorophyll fluorescence

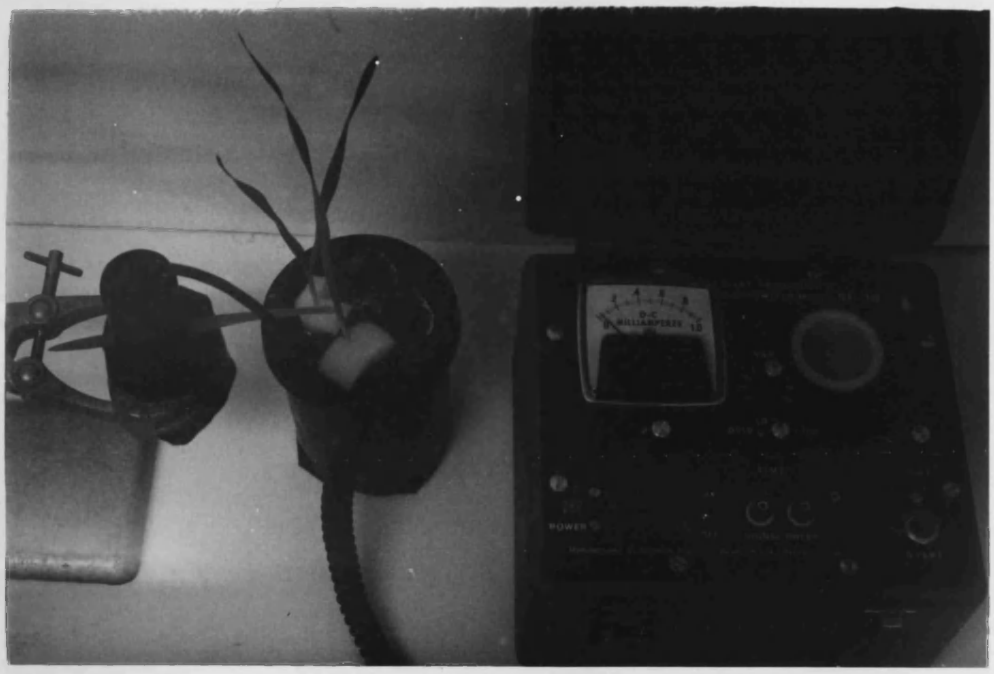
Isoproturon was added to the root solution of 14 day old plants grown in liquid culture. After 24 h, the herbicide was replaced by fresh nutrient solution while roots were rinsed in distilled water for 2 min. Fluorescence readings were taken at commencement and every 24 h for 7 days.

Plants were maintained in darkness for 1 h prior to measurement, and further operations were carried out under green safety lamps. A previously marked out 2 mm area on the adaxial

surface, 4 cm from the tip of leaf 1, was subjected to illumination from the probe of a plant productivity fluorometer, model 'SF-10' (Richard Brancker Research Ltd., Ottawa, Canada) (Plate 7.1). The portable Kautsky apparatus incorporated a photodiode sensor and a light emitting diode (LED), which was used to provide monochromatic illumination at a maximum of 670 nm, with a light intensity of 3.25 $\mu E\ m^{-2}s^{-1}$ for periods of 10 sec duration. The apparatus was connected to an 'MSE Fisons Vitatron' chart recorder which measured fluorescence yield in six separate leaves at each assessment period. Two arbitrary values, ΔF and FI/FP ratios were calculated from the fluorescence curves. ΔF represents the difference in fluorescence yield between the peak (FP) and 10 seconds (FT) after a dark-light transient(↑) (Figure 7.1a). ΔF is increasingly lowered in plants with inhibited photosynthesis (Fedtke and Schmidt, 1983). In untreated chloroplasts, the peak (FP) declines due to reoxidation of the primary PSII electron acceptor, Q_A and redistribution of energy from the strongly fluorescent photosystem II to the weakly fluorescent photosystem I. In treated plants, a smaller decline is expected, since electron flow through PSII to PSI is blocked by the herbicide. FI/FP represents the rise in fluorescence between the intermediate level (FI) and the peak (FP), expressed as a ratio. Reliability of the FI/FP ratio depends mainly on the constancy of the origin (O) level (Figure 7.1b). FO → FI indicates that charge separation in PS II is taking place (Papageorgiou, 1975). FI → FP represents the chlorophyll fluorescence of variable yield, which is sensitive to rates of electron transport and thylakoid ultra-structure. The change FI → FP is correlated with the net reduction of the electron acceptor, Q_A, using reductants generated by water

PLATE 7.1

The plant productivity fluorometer used in section 7.2.2.


D.C.
MILLIAMPERES
POWER

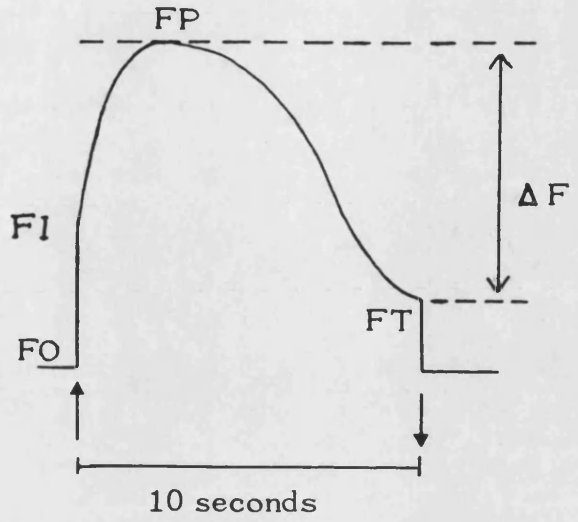


FIGURE 7.1a

Hypothetical curve to calculate ΔF. (after Fedtke and Schmidt, 1983).

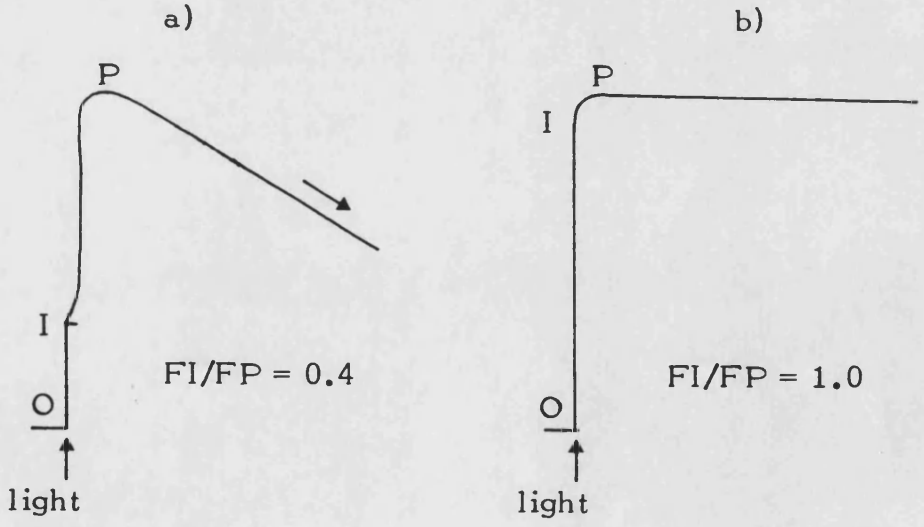


FIGURE 7.1b

Hypothetical curve to calculate FI/FP a) untreated and b) treated with herbicide. (after Cadahia et al., 1982).

splitting (Lavorel and Étienne, 1971). Thus a high FI/FP ratio suggests little reduction of the electron acceptor. A decrease in the value FI/FP suggests the reappearance of previously inhibited photosystem II photochemical centres in tolerant species.

7.2.3 Chloroplast isolation

Leaves from 14 day old plants were ground in a pestle and mortar in grinding medium (0.5 M sucrose; 0.05 M $KHCO_3$ to pH 7.5) and strained through two layers of muslin into cooled centrifuge tubes. Tubes were balanced and spun in an 'MSE high speed 18' centrifuge at 3°C for 90 sec at 1,000 rpm, then the pellet discarded. The supernatant was spun at 8,000 rpm for 12 min to remove mitochondria. The resulting supernatant was poured off and the pellet resuspended in an aliquot of grinding medium. Suspensions were kept at 0 - 3°C throughout the procedure and stored in an ice box until use.

7.2.4 Chlorophyll determination

The chlorophyll content of preparations was determined spectrophotometrically by the Arnon (1949) modification of the method of Mackinney (1941). A 0.25 cm^3 sample of chloroplast suspension was spun at 3,000 rpm for 10 min with 4.75 cm^3 of 80% acetone. The optical density of this solution was measured at 645 nm and 663 nm in a 'Shimadzu UV-260' ultraviolet spectrophotometer, using 80% acetone as a blank. The concentration of chlorophyll in acetone was calculated using the formula:-

$$\text{chlorophyll concn. in acetone } (g\ cm^{-3}) = 20.2 \times A_{645} + 8.02 \times A_{663}$$

Values were adjusted to obtain the amount of chlorophyll per cm^3.

7.2.5 The oxygen electrode

The apparatus consisted of a 'Hansatech' oxygen electrode attached to a 'Churchill' chiller thermocirculator maintained at 20°C. This was calibrated by pipetting 3 cm^3 of distilled water into the electrode chamber and adding a few grains of sodium dithionite ($Na_2S_2O_4$ + H_2O) to deoxygenate the system. The magnitude of the deflection on the attached chart recorder was calibrated in terms of µmoles O_2 evolved, since 1 litre of distilled water at 20°C contains 280 µmoles O_2.

To follow photosystem II activity, 1.9 cm^3 of electron transport buffer (2.5 x 10^{-2}M PO_4^-; 10^{-2}M NH_4Cl; 6 x 10^{-4}M NaN_3 at pH 8) (Percival and Dodge, 1983), was added to the reaction chamber after thorough rinsing with distilled water. 0.3 cm^3 of 10^{-4}M paraquat and 0.5 cm^3 of the chloroplast suspension were pipetted into the chamber and the volume made up to 3.0 cm^3 with either distilled water (as a control) or a known concentration of isoproturon solution. The chamber was covered with a black cloth for 2 min, prior to illumination from a 'Kershaw' daylight strip projector, with a 500 watt tungsten bulb, at 28 cm distance from the chamber.

The deflection was recorded on a 'Vitatron' chart recorder set at 20 mV and 10 mm min^{-1}. The rate of electron transport in the suspension was calculated using the formula:-

$$\text{Oxygen evolution } \mu\text{moles } \tfrac{1}{2}O_2 mg^{-1}h^{-1} = \frac{\text{deflection measured in units} \times \frac{60}{\text{(h)}} \times \text{value of 1 unit} \div \frac{2.5}{\text{(mV)}} \div 1000}{\text{chlorophyll concn. } (cm^3)}$$

A range of isoproturon concentrations was used and each treatment

replicated three times. The inhibitory potency value (I_{50}) for each species, which is the concentration required for 50% inhibition of chloroplast electron transport, was calculated by regression analyses of inhibition induced by various concentrations of isoproturon.

7.3 RESULTS

7.3.1 The effect of isoproturon on photosynthetic rates

7.3.1.1 Carbon dioxide uptake

Figures 7.2, 7.3 and 7.4 show the rate of carbon dioxide uptake by the three species, expressed as a % of the control at time 0, following treatment with isoproturon to the root solution at 0.01 mM, 0.1 mM and 0.5 mM respectively.

At the lowest concentration of isoproturon,photosynthetic rates of *B. sterilis* plants remained virtually unchanged over the assessment period. *B. willdenowii* plants were partially inhibited, but recovery of photosynthesis was complete by the fourth day after treatment. Rates in barley seedlings fluctuated over the 8 day period, but alterations in the rate of carbon dioxide uptake were negligible.

A concentration of 0.1 mM inhibited the rate of photosynthesis in *B. sterilis* by as much as 90% after 24 hours. Recovery after herbicide removal was slow, but by the 8th day after treatment, plants had attained 70% of the initial rate. Photosynthesis totally ceased in *B. willdenowii* but resumed two days after isoproturon removal, and followed a similar increase to *B. sterilis*. Barley plants initially suffered decreased rates, though recovery was rapid and complete by the 4th day after removal.

Figure 7.4 shows that at a concentration of 0.5 mM, *B. sterilis* did not recover from 100% inhibition of photosynthesis, and plants eventually died. At the same concentration, carbon dioxide uptake in *B. willdenowii* was similarly reduced by 100%, but slow recovery ensued. Barley plants maintained a steady increase in photosynthetic capacity after day 3.

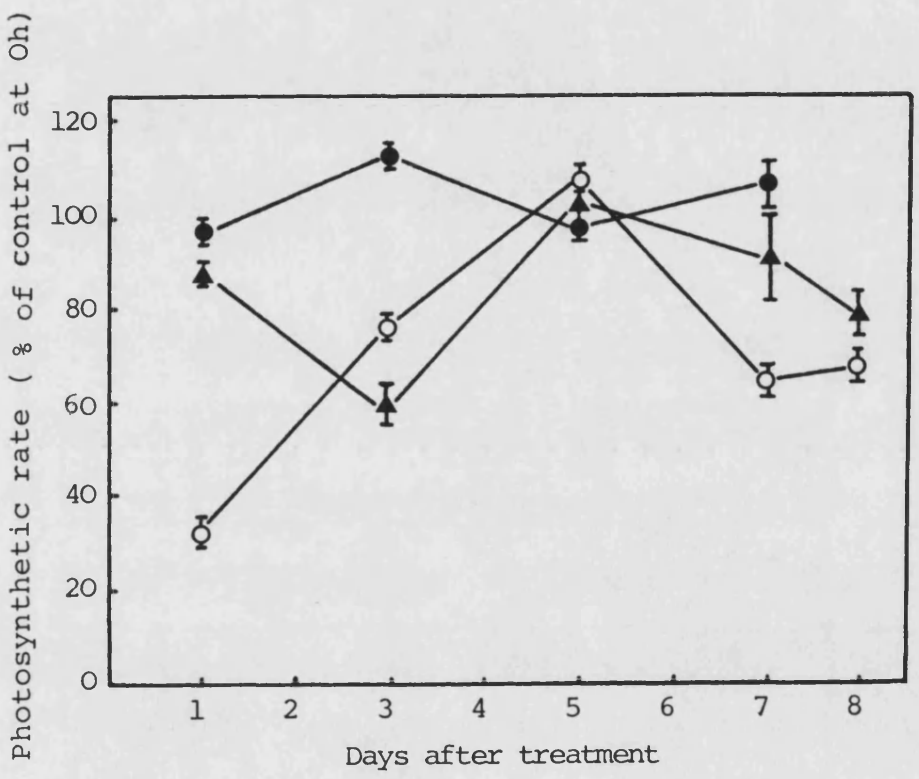


FIGURE 7.2

Recovery from photosynthetic inhibition following removal of 0.01 mM isoproturon from the root solution at day 1. *B. sterilis* (●), *B. willdenowii* (O) and barley (▲).

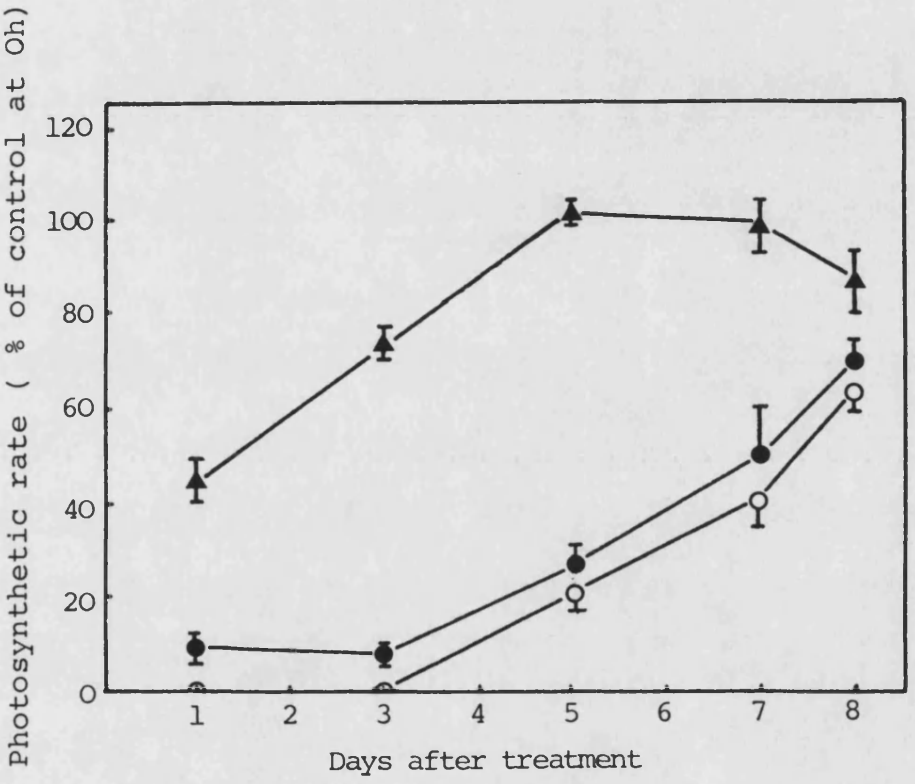


FIGURE 7.3

Recovery from photosynthetic inhibition following removal of 0.1 mM isoproturon from the root solution at day 1. *B. sterilis* (●), *B. willdenowii* (O) and barley (▲).

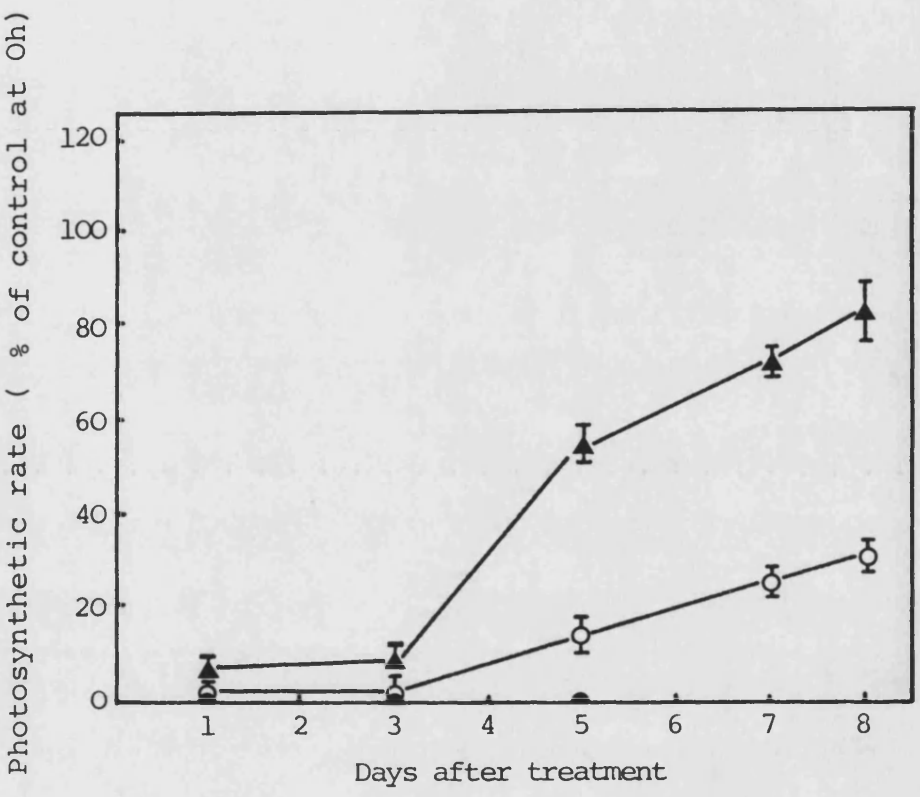


FIGURE 7.4

Recovery from photosynthetic inhibition following removal of 0.5 mM isoproturon from the root solution at day 1. *B. sterilis* (●), *B. willdenowii* (O) and barley (▲).

7.3.1.2 Fluorescence yields

The arbitrary unit of fluorescence, ΔF, is expressed as a % of ΔF in untreated plants in Figures 7.5, 7.6 and 7.7. Figure 7.5 represents the leaf fluorescence kinetics when plant roots were subjected to a 0.001 mM concentration of isoproturon for 24 hours at day 0. Slightly decreased values of ΔF resulted from isoproturon application to all three species, though they recovered within 48 hours. Fluorescence kinetics of *B. willdenowii* plants were variable over the 7 day period.

B. sterilis and *B. willdenowii* plants responded similarly when higher concentrations of isoproturon were applied to the roots. ΔF was relatively steady over the assessment period, yet increasingly reduced by 0.01 and 0.1 mM isoproturon. At neither concentration did recovery of ΔF to its original value occur. On the contrary, treated barley plants showed a rapid recovery well past the original value of untreated plants, though they were initially affected more significantly than the *Bromus* spp.

The FI/FP ratio is used to measure effects of the herbicide on chlorophyll fluorescence during the first millisecond after illumination. A value of 1.0 indicates total inhibition of electron transport. Figures 7.8, 7.9 and 7.10 indicate the FI/FP ratios after treatment with 0, 0.01 and 0.1 mM isoproturon respectively. Control values fluctuated between 0.45 and 0.65 depending upon species, *B. sterilis* being intermediate, between barley at around 0.6, and *B. willdenowii* at 0.5. There was a slight increase in the ratio over the 7 day period for all species.

At a 0.01 mM concentration of isoproturon, *B. sterilis* remained fairly constant at 0.6, while the FI/FP ratios of *B. willdenowii* and

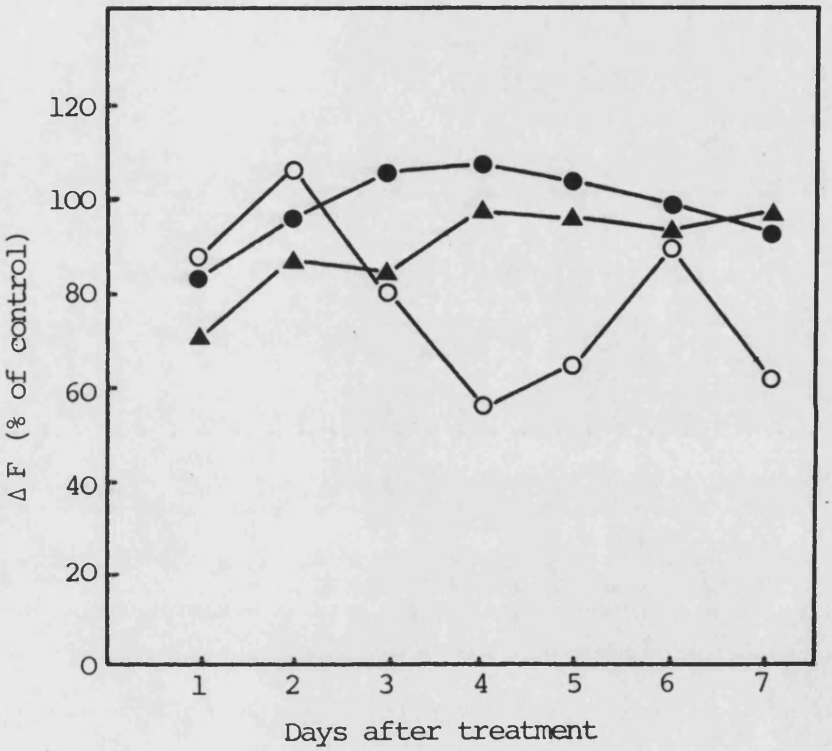


FIGURE 7.5

Chlorophyll fluorescence kinetics following root applications of 0.001 mM isoproturon until day 1. *B. sterilis* (●), *B. willdenowii* (O) and barley (▲).

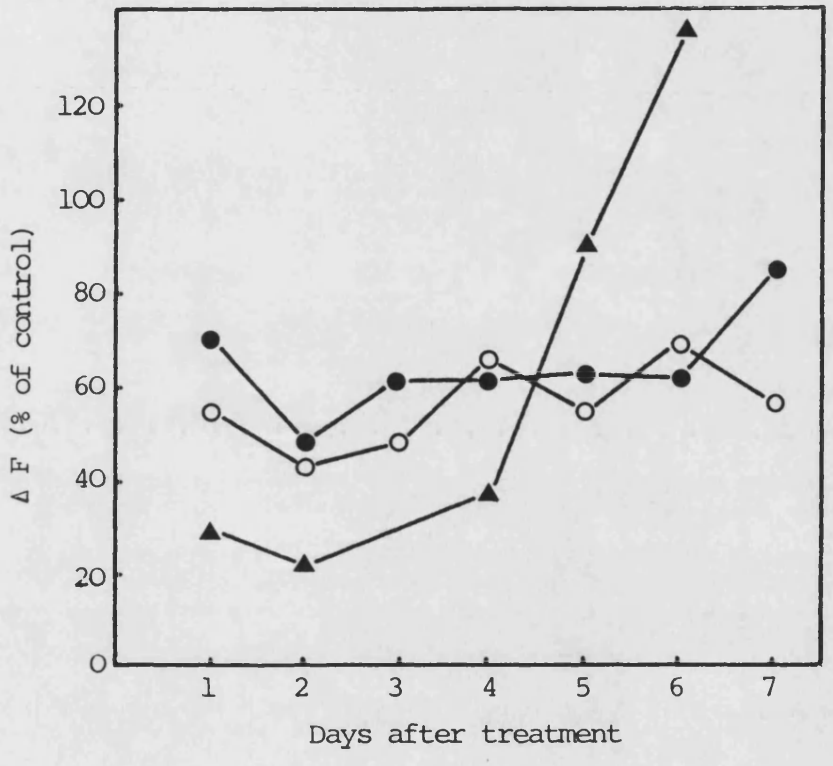


FIGURE 7.6

Chlorophyll fluorescence kinetics following root applications of 0.01 mM isoproturon until day 1. *B. sterilis* (●), *B. willdenowii* (O) and barley (▲).

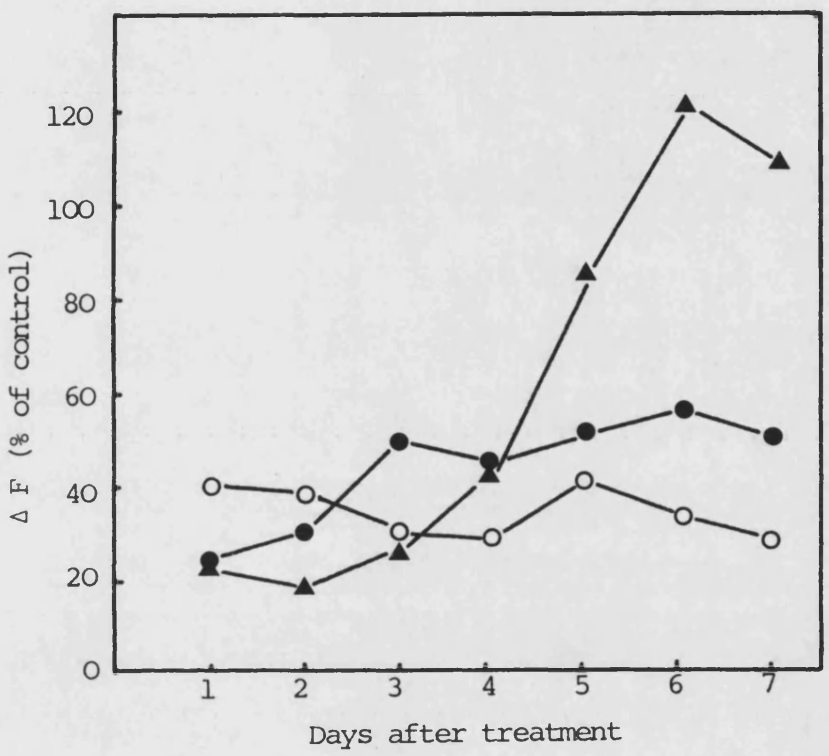


FIGURE 7.7

Chlorophyll fluorescence kinetics following root applications of 0.1 mM isoproturon until day 1. *B. sterilis* (●), *B. willdenowii* (O) and barley (▲).

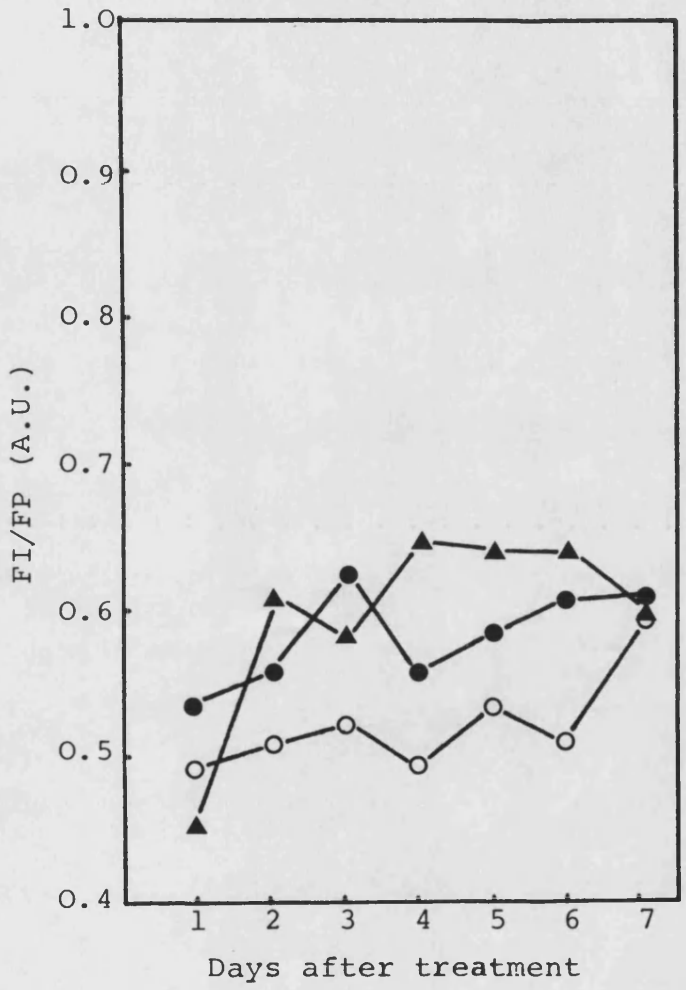


FIGURE 7.8

Chlorophyll fluorescence kinetics of untreated plants over a 7 day period.

B. sterilis (●), *B. willdenowii* (○) and barley (▲).

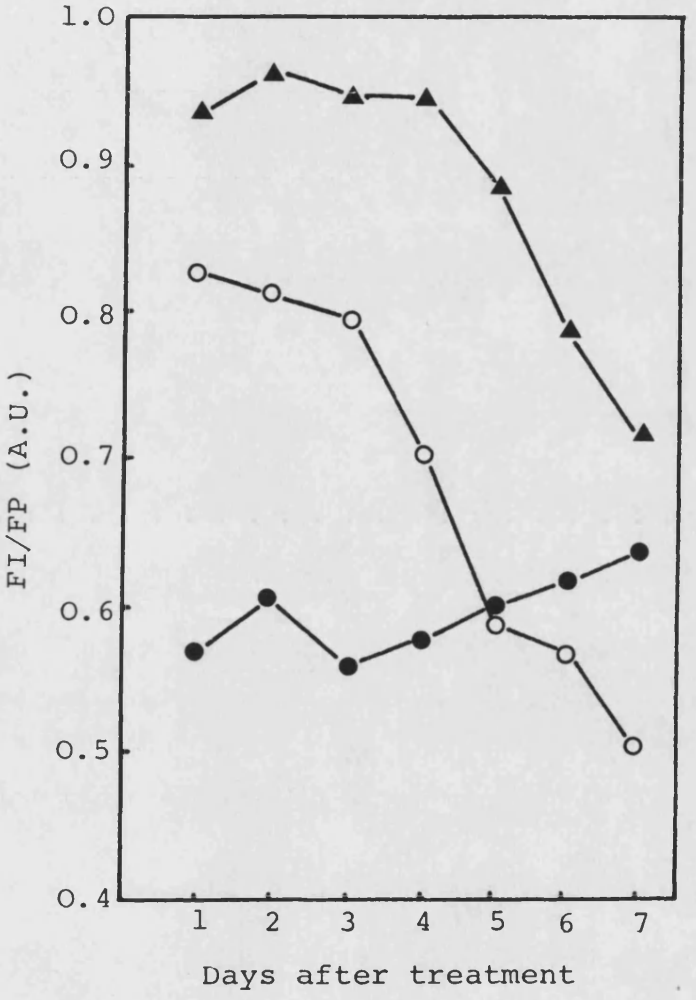


FIGURE 7.9

Chlorophyll fluorescence kinetics following root applications of 0.01 mM isoproturon until day 1.

B. sterilis (●), *B. willdenowii* (O) and barley (▲).

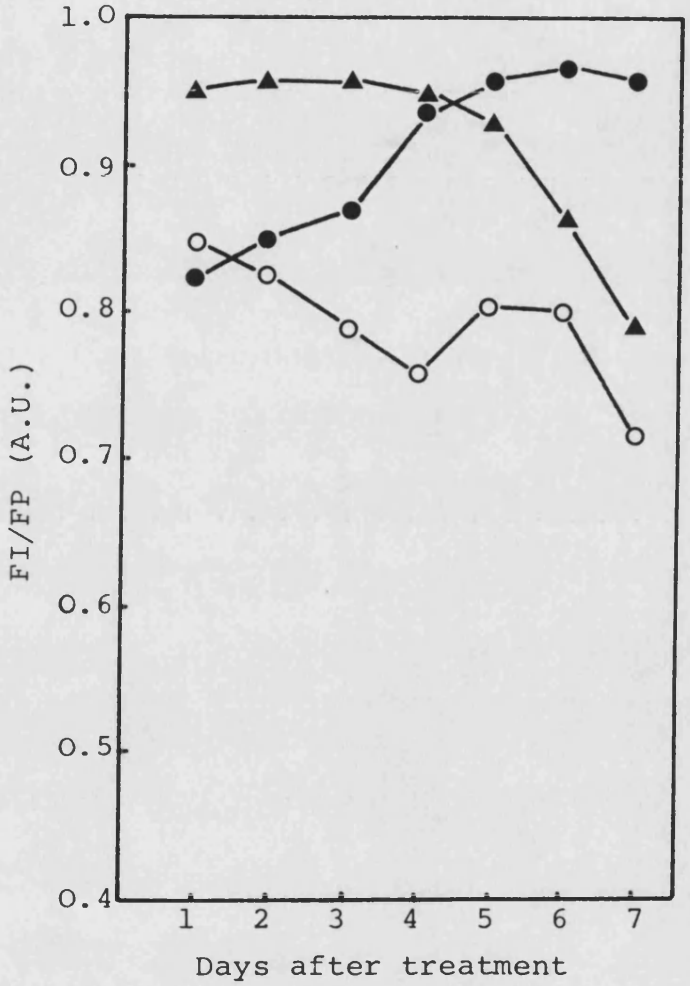


FIGURE 7.10

Chlorophyll fluorescence kinetics following root applications of 0.1 mM isoproturon until day 1.

B. sterilis (●), *B. willdenowii* (O) and barley (▲).

barley plants rapidly declined following initial stimulation. At the higher concentration of 0.1 mM, *B. sterilis* plants were also affected, the FI/FP ratio increasing to 0.96 over the 7 day period. Although the values for *B. willdenowii* and barley were initially greater than for *B. sterilis*, they decreased by 1.4 and 1.6 units respectively to 0.71 and 0.79.

7.3.2 The effectiveness of *in vitro* chloroplast electron transport inhibition by isoproturon

Table 7.1 shows the I_{50} values of isoproturon for the three species (the concentration required to inhibit chloroplast electron transport by 50%). These were calculated by regression analyses of inhibition by various concentrations of isoproturon.

The I_{50} values of *B. sterilis* and barley chloroplasts were virtually identical, with small standard errors of the mean. *B. willdenowii* appeared to require larger doses of isoproturon to inhibit electron transport, but the standard error was large and brought the range between 2.8×10^{-7}M and 1.6×10^{-6}M.

TABLE 7.1

Inhibition of electron transport (I_{50} value) in chloroplasts isolated from 14 day old plants of *B. sterilis*, *B. willdenowii* and barley, following *in vitro* treatment with isoproturon.

species	concentration (M)	SE
B. sterilis	2.3×10^{-7}	1.0×10^{-7}
B. willdenowii	9.4×10^{-7}	6.6×10^{-7}
barley	2.0×10^{-7}	6.4×10^{-8}

7.4 DISCUSSION

There are few reports on the selectivity mechanisms of isoproturon, although much has been published on the phenylurea herbicides. In general, selectivity of this class of compounds appeared to be associated with differential sensitivity of the photosynthetic mechanism (Hilton *et al.*, 1963; Ashton, 1965; Ashton *et al.*, 1977).

i) The cessation of CO_2 uptake following herbicide addition indicates a rapid uptake and binding of isoproturon to the inhibitor site within the chloroplasts. At low concentrations, photosynthesis was not totally inhibited, leaving a residual electron flow for $NADP^+$ reduction. After herbicide removal, the recovery rate of CO_2 uptake was species-dependent. However, rates can only reasonably be compared when they produce a similar initial degree of inhibition, as in Figure 7.4. Van Oorschot (1968) was able to make comparisons by removing the herbicide from the root environment at a certain level of inhibition of photosynthesis, and following CO_2 uptake during a subsequent period. However, without continuous monitoring of photosynthesis, it is difficult to assess the point at which to remove the herbicide. In any case, this method overlooks the relative intensities of inhibition produced by each species at a given concentration, which may reflect upon differential uptake and translocation. Van Oorschot (1965) found recovery from monuron inhibition in plantain *(Plantago* spp.) but not in maize, and concluded that the tolerant plantain species promoted monuron inactivation. From the results of this present study it appeared that barley plants could inactivate isoproturon, thus resuming their

original rate of CO_2 uptake and subsequent fixation. *B. willdenowii* plants continued to photosynthesise at a reduced rate, suggesting that the rate of metabolic breakdown of isoproturon by this species is slow. Differential rates of breakdown have been proposed to explain differences in susceptibility (Swanson and Swanson, 1968; Müller and Sanad, 1975; Müller *et al.*, 1977). Varietal differences in the rate of metoxuron degradation in wheat, primarily through N-demethylation, partially accounted for selectivity (van Leewen and van Oorschot, 1976), though no such differences were apparent for isoproturon (Müller *et al.*, 1977). However, this does not eliminate the possibility of variations in degradation rates between plants of different species. The inability of *B. sterilis* plants to recover from 100% inhibition suggests that they cannot metabolise isoproturon. Their recovery from lower doses occurred as a result of herbicide removal.

The capacity for detoxification in itself is not sufficient for the determination of sensitivity or tolerance, but should be seen together with the rates of uptake and translocation. In the soil, actual concentrations of herbicide in the root zone will be considerably lower than in nutrient solution, thus the rate of inactivation may keep pace with the rate of uptake (van Oorschot, 1968). This may explain why *B. willdenowii* was relatively tolerant to isoproturon in pot trials, yet susceptible in nutrient culture.

ii) Chlorophyll fluorescence represents a meaningful probe for testing interference with the redox state of the PS II complex (van Assche and Carles, 1981). Kinetics followed a similar pattern to CO_2 uptake assays, although the former measured a more immediate

response of inhibition. Electron transport inhibition eliminates the slow decline in fluorescence after the peak (FP), thus a low ΔF value indicates reduced photosynthesis. The much larger initial decrease in ΔF of barley plants at a concentration of 0.01 mM isoproturon suggests that the herbicide is reaching the site of action at a comparable rate to the other species, hence uptake may not be limiting effects at the chloroplast level. The increase in ΔF over time as exemplified by barley plants, suggests a resumption of the decline in fluorescence as oxidants generated by electron flow mediated by PS I, and by reduction of CO_2 exert an effect (Critchley and Smillie, 1981). The relatively constant values of ΔF in the *Bromus* spp. over the 7 days indicated continued inhibition of electron transport, allowing limited production of NADPH.

The rise FI → FP reflects the transient net reduction of the electron acceptor Q_A using reductants generated by water splitting (Lavorel and Étienne, 1971). Fluorescence kinetics of untreated plants fluctuated over the 7 days, increasing slowly with time. This may relate to less efficient reduction with increasing age of the plants, though Cadahia *et al.* (1982) found FI/FP ratios practically unaffected by plant age. *B. willdenowii* plants appeared to have a lower fluorescence yield, which would have resulted from a slower charge separation in PS II occurring upon illumination (Papageorgiou, 1975). In the present investigation, the FI/FP ratio of *B. sterilis* plants gradually increased, even after herbicide removal, suggesting that this species is incapable of detoxifying isoproturon. It is interesting to note that isoproturon had a greater initial effect upon chlorophyll fluorescence in barley plants than in *B. sterilis*, although the steep decline in FI/FP over

time indicated a recovery of electron flow. This suggests that detoxification was slow compared to transport of isoproturon into the chloroplasts (Voss *et al.*, 1984), and supports the previous conclusion that barley possesses a detoxification mechanism. It appears too, that *B. willdenowii* can metabolise the herbicide since less energy is dissipated as fluorescence over time.

Problems associated with obtaining reproducible induction curves may arise if the apparatus cannot detect the FO level of fluorescence. A transient recorder will ensure a standardised base level for subsequent photochemical rise assessments. A sufficient dark adaptation period prior to measurements is required, presumably to establish the dark redox state of the plastoquinone pool which affects the redox state of Q_A (Voss *et al.*, 1984).

iii) The similarity in I_{50} values of *B. sterilis* and barley plants implies that these species did not differ with respect to the inherent sensitivity of their chloroplast membranes to isoproturon. The greater value of 9.4×10^{-7}M for *B. willdenowii* plants may account for the greater tolerance of this crop in pot trials. The difference cannot be attributed to variations in the test method used, a problem associated with the comparison of I_{50} values from the literature (Dicks, 1978). Dicks (1978) reported a value of 1.7×10^{-7}M for isoproturon, although chloroplast sources differed.

There are limitations which must be recognised when investigating the action of herbicides via the use of isolated chloroplasts. The I_{50} value only measures the response to a herbicide concentration in the reaction mixture, and not to the amount of inhibitor inside the chloroplast, which is much smaller

(Wessels and van der Veen, 1956). The value gives no indication of the herbicidal efficiency under *in vivo* conditions when the herbicide may fail to accumulate at specific sites of action, and thus may not be available. Santakumari and Das (1978) stressed the importance of correct interpretation of results, since Hill reaction activity varied between almost none on herbicide sprayed plants, to severe inhibition of chloroplasts treated *in vitro*.

8. HERBICIDE METABOLISM

8.1 INTRODUCTION

Differential metabolism, both qualitative and quantitative, can contribute to the selectivity of herbicides, and in several notable cases, such differences are considered to form the primary basis of selectivity (Geissbühler *et al.*, 1975b). Oxidative N-demethylation has been recognised as an important reaction for the detoxification of substituted phenylurea herbicides (Figure 1.7). The initial product of N-demethylation, the hydroxymethyl intermediate, retains significant phytotoxicity until glucosidic conjugates are formed (Geissbühler and Voss, 1971). Additionally, N-monomethyl metabolites are usually less phytotoxic than their dialkylated parent compounds and phytotoxicity disappears completely with the second demethylation step (Smith and Sheets, 1967). N-demethylation may be followed by hydrolysis of the dealkylated ureas to the corresponding anilines. However, the amount of free anilines detected have been consistently small (Geissbühler *et al.*, 1963b). Another major degradative reaction has been demonstrated for certain phenylureas, namely ring hydroxylation followed by glucosylation (Ryan *et al.*, 1981). In tolerant cereal species, ring-methyl oxidation of chlortoluron predominated over N-demethylation, whereas in the susceptible cereal weeds, the latter was a more important pathway (Ryan, 1981). All products of ring-methyl oxidation are essentially non-phytotoxic, thus predominance of this pathway may offer an explanation for the enhanced tolerance of some species to chlortoluron (Ryan *et al.*, 1981; Ryan and Owen, 1982). The relative contribution of each pathway to degradation varied between species and among varieties of the same species (Ryan and Owen, 1983). The

metabolic pathways of isoproturon degradation in plants have not been fully elucidated.

Differential detoxification rates in tolerant and sensitive species may also explain susceptibility to herbicides (Rogers and Funderburk, 1967; Smith and Sheets, 1967; Swanson and Swanson, 1968). Crop tolerance has been associated with rapid inactivation, but the speed may vary between cultivars, as van Leewen and van Oorschot (1976) have shown with metoxuron for wheat varieties. Müller *et al.* (1977) could find no differences in isoproturon degradation rates between varieties of wheat and barley, the majority showing tolerance.

Experiments were designed to elucidate whether detoxification processes could explain differences in selectivity between *B. sterilis*, *B. willdenowii* and barley:-

i) thin-layer chromatographic techniques were used to demonstrate the detoxification products of ^{14}C-isoproturon. R_{ip} values were calculated to compare the relative number of products of ^{14}C-isoproturon. Densitometer scans of TLC autoradiographs were used to compare the location and size of metabolites.

8.2 MATERIALS AND METHODS

8.2.1 Thin-layer chromatography

Eight plants of each test species were treated for 7 days with ^{14}C-isoproturon via the nutrient solution (0.17 K Bq cm^{-3}; 4.6 x 10^{-3} μ Ci cm^{-3}). At harvest, each batch of eight plants was extracted four times with methanol/water (8:2v/v) at room temperature. Tissue was ground in a pestle and mortar containing methanol/water and filtered through 'Whatman grade C' glass fibre filter paper, using a vacuum attached to a Büchner funnel. The resulting methanol/water solution was evaporated using a 'Büchi Rotavapor-R' (Orme Scientific Ltd., Manchester, U.K.) connected to an 'Edwards Speedivac ED-35 pump' (Edwards High Vacuum Ltd., Crawley, U.K.). The aqueous phase, remaining after methanol evaporation, was successively extracted with hexane (25 cm^3), methylene chloride (35 cm^3) and ether (20 cm^3), using 50 cm^3 separating funnels. Each fraction was evaporated to dryness at laboratory temperature in a 50 cm^3 round-bottomed flask attached to the rotary evaporator. The extracted samples were then redissolved in 0.25 cm^3 of the respective solvents and chromatographed separately along with the final aqueous phase.

The extracts were applied to pre-coated silica gel $60F_{254}$ aluminium sheets (Merck, Darmstadt, Germany), 1 cm from the bottom edge. Each spot contained 100 μℓ of the respective extract, and a standard of ^{14}C-isoproturon with the unlabelled product was spotted at 30 μℓ. When completely dry, the plate was placed in a previously equilibrated tank containing chloroform/ethanol (9:1v/v) developing solvent. The plates were marked at 1 cm from the top, and removed when the solvent front had reached this mark.

Autoradiographs of the TLC plates spotted with extracts from ^{14}C-isoproturon treated plants were produced by exposure to sheets of 'Kodak Industrex type CX2' X-ray film for a period of 14 days. These were developed as described previously.

8.2.2 Measurement of R_{ip} values

The distance from the base line on the TLC autoradiograph to the centre of each radioactive spot was divided by the distance from the base to the spot representing non- degraded ^{14}C-isoproturon (standard). Any similarity in the location of a spot present on different plates was denoted by a common letter in parentheses following the R_{ip} value.

8.2.3 Densitometer scans

Autoradiographs of TLC plates were placed in a 'Joyce-Loebl chromoscan 3' which was set at a scan length of 175 mm and an aperture width of 0.3 mm. The density of spots relative to a maximum absorbance of 1.0 was shown for each fraction.

8.3 RESULTS

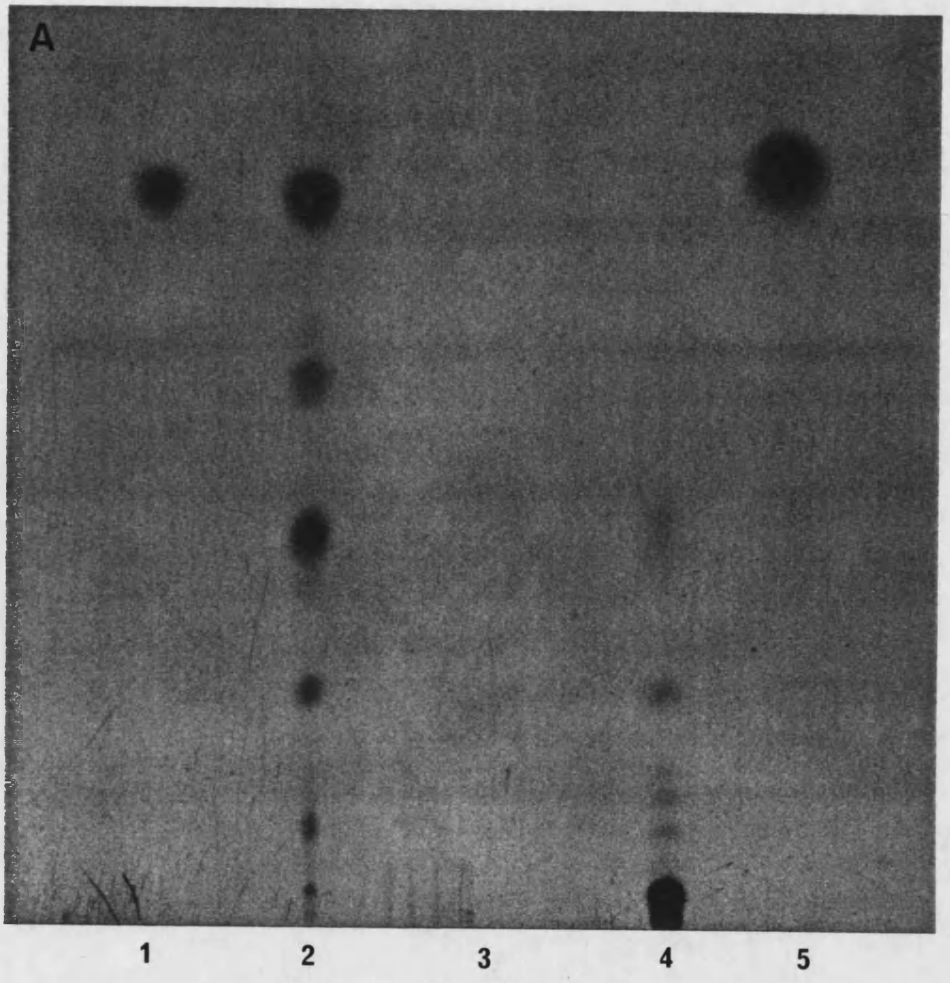
8.3.1 The effectiveness of isoproturon degradation

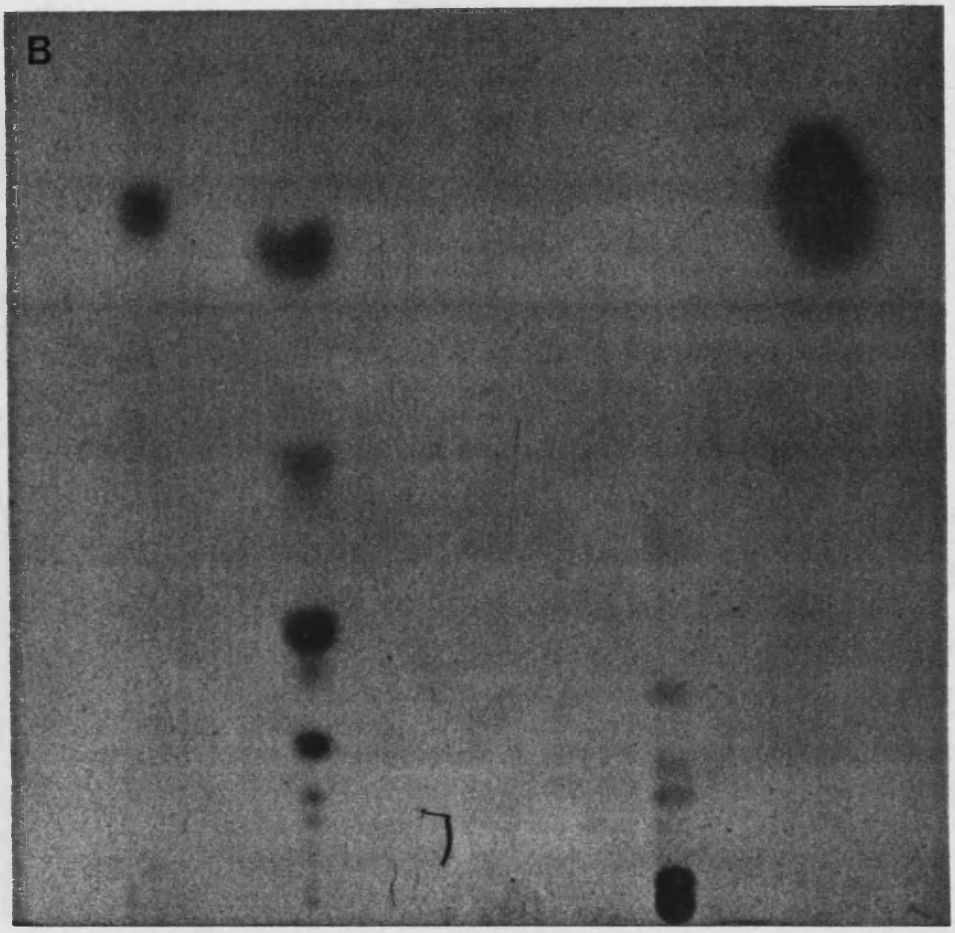
Radioassay of the different fractions separated by TLC was carried out by autoradiography, and expressed in Plates 8.1 A-C. Figure 8.1 shows a tracing of the TLC autoradiographs of each species for easy comparison. The R_{ip} value of each spot is shown in Table 8.1. Values of 0.95 - 1.0 were considered to represent the parent compound. The hexane extractable phases of the *Bromus* spp. contained only one radioactive metabolite each, with an R_{ip} value of 0.96 and 0.95 respectively. One extra product (R_{ip} = 0.68) partitioned into hexane in barley plants. A number of metabolites were detected in the methylene chloride fraction of all three species. Barley contained additional products to those recorded for *B. sterilis* and *B. willdenowii*. The R_{ip} values for metabolites from *B. sterilis* showed little similarity to the values of the other species. No detectable radioactivity was found in the ether phase of any species. The aqueous fraction of each species contained five common metabolites, and although at a different location, *B. sterilis* and barley each possessed an additional product. The parent compound was not present in the aqueous fraction of any species.

Figures 8.2 a-d show the densitometer scans produced from TLC autoradiographs, each representing a particular fraction. Figure 8.2a indicates the location of the parent compound, which was also present in varying quantities in the hexane and methylene chloride fractions. There appeared to be a greater quantity of the non-degraded ^{14}C-isoproturon in barley extracts in both these fractions. The additional product (R_{ip} = 0.68) in barley was only present in small amounts. In the methylene chloride fraction, barley plants

PLATE 8.1

Autoradiographs of TLC plates treated with plant extracts from ^{14}C-isoproturon-treated seedlings of A) *B. sterilis*, B) *B. willdenowii* and C) barley. Columns are labelled to represent the various fractions 1) hexane, 2) methylene chloride, 3) ether, 4) aqueous and 5) standard parent compound.


A
1
2
3
4
5


B
1
2
3
4
5

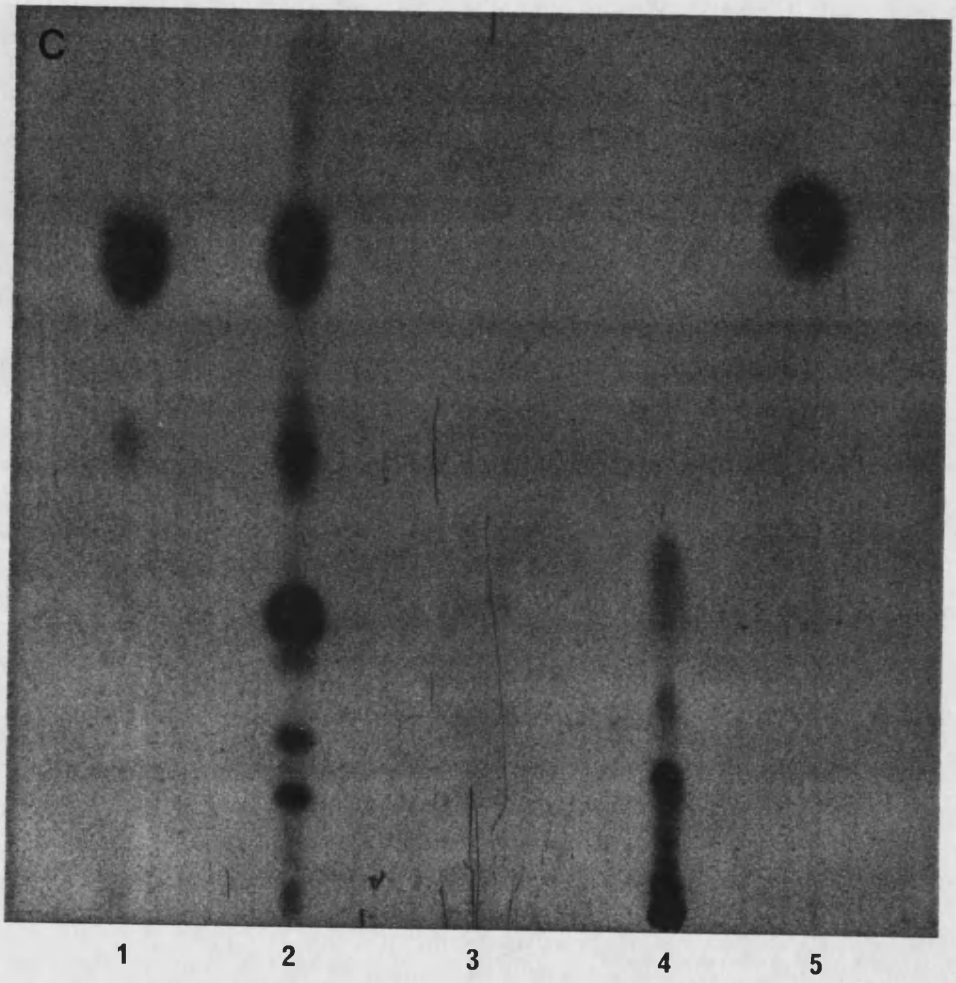

C
1
2
3
4
5

FIGURE 8.1

Tracing of TLC autoradiographs of A) *B. sterilis*, B) *B. willdenowii* and C) barley to show the location of radioactive metabolites.

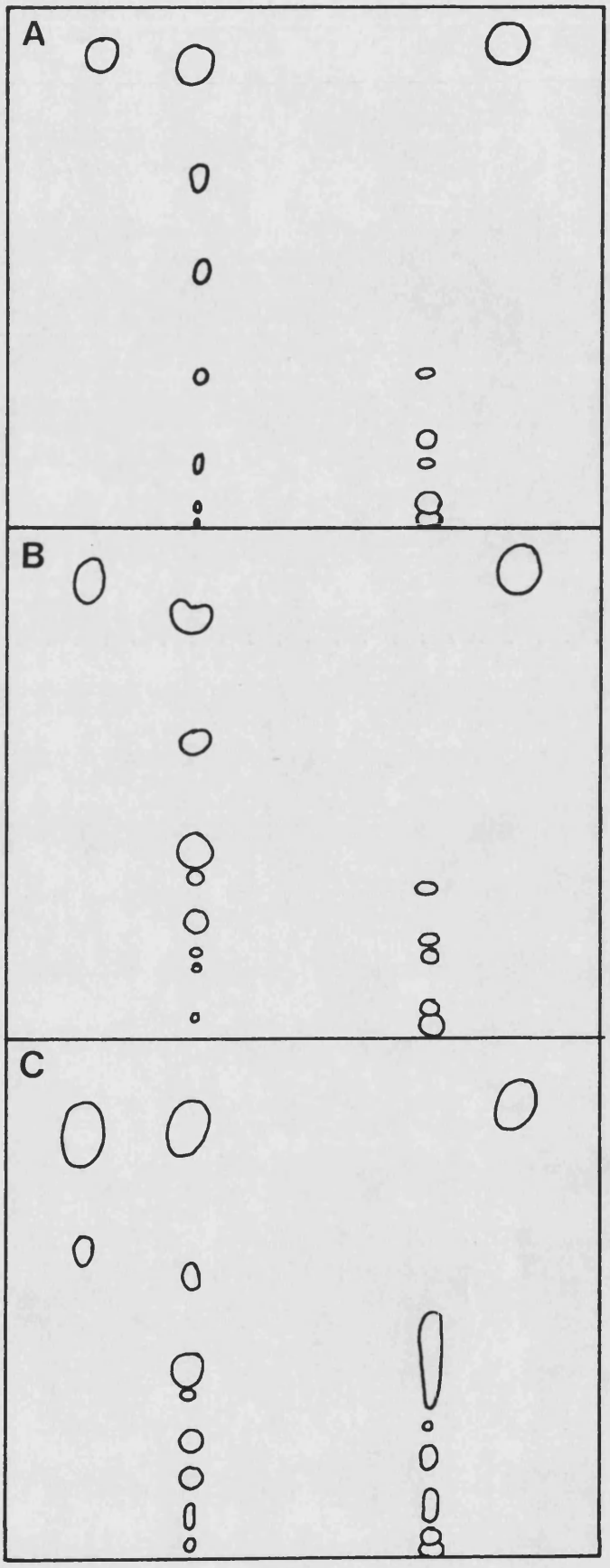

A
B
C

TABLE 8.1

R_{ip} values of isoproturon and its metabolites.

species	fraction hexane	methylene chloride	aqueous
B. sterilis	0.96 (a)	0.96 (a)	0.29 (g)
		0.71 (b)	0.19 (h)
		0.50	0.15
		0.29 (d)	0.11 (i)
		0.12 (f)	0.03 (k)
		0.03	0.0 (l)
B. willdenowii	0.95 (a)	0.90 (a)	0.29 (g)
		0.61	0.19 (h)
		0.38 (c)	0.14 (i)
		0.32	0.03 (k)
		0.23 (d)	0.0 (l)
		0.15 (e)	
		0.12 (f)	
barley	0.94 (a)	0.95 (a)	0.45
	0.68	0.72 (b)	0.30 (g)
		0.65	0.20 (h)
		0.44	0.11 (i)
		0.37 (c)	0.05 (k)
		0.26 (d)	0.0 (l)
		0.17 (e)	
		0.0	

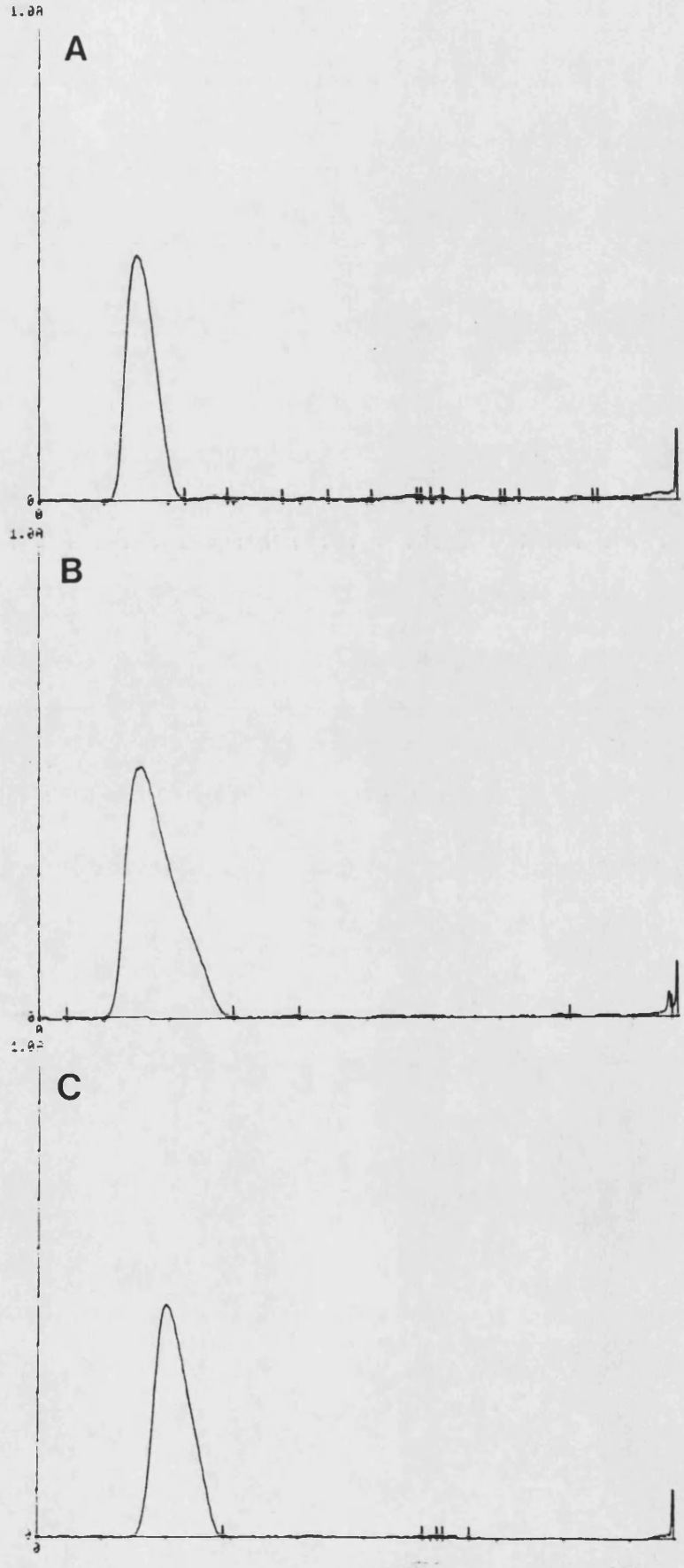
possessed the largest quantities of all the metabolites present. This was also the case, with one exception (R_{ip} = 0.03), for the aqueous fraction. At this location, the metabolite was of the largest quantity in *B. sterilis* plants.

FIGURE 8.2

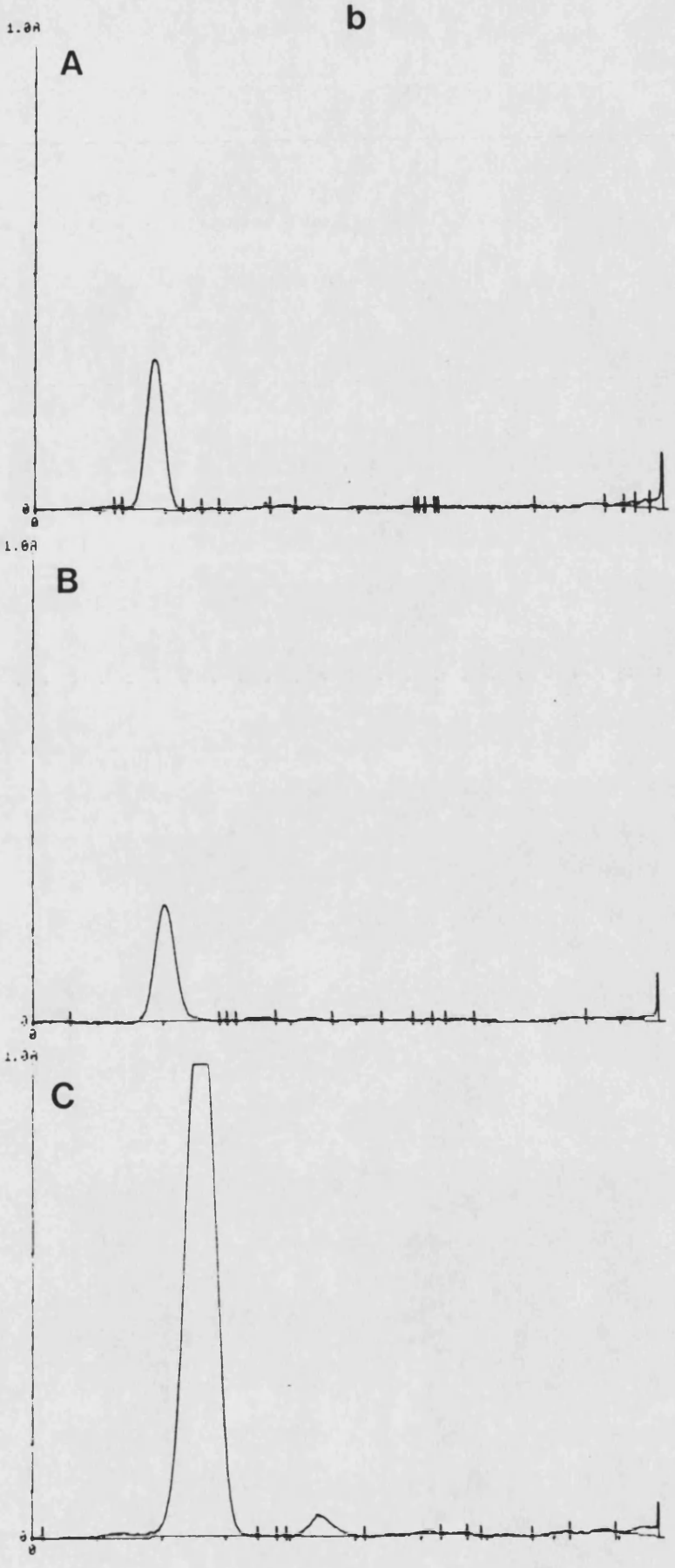
Densitometer scans of TLC plates treated with plant extracts from ^{14}C-isoproturon-treated seedlings of A) *B. sterilis*, B) *B. willdenowii* and C) barley.

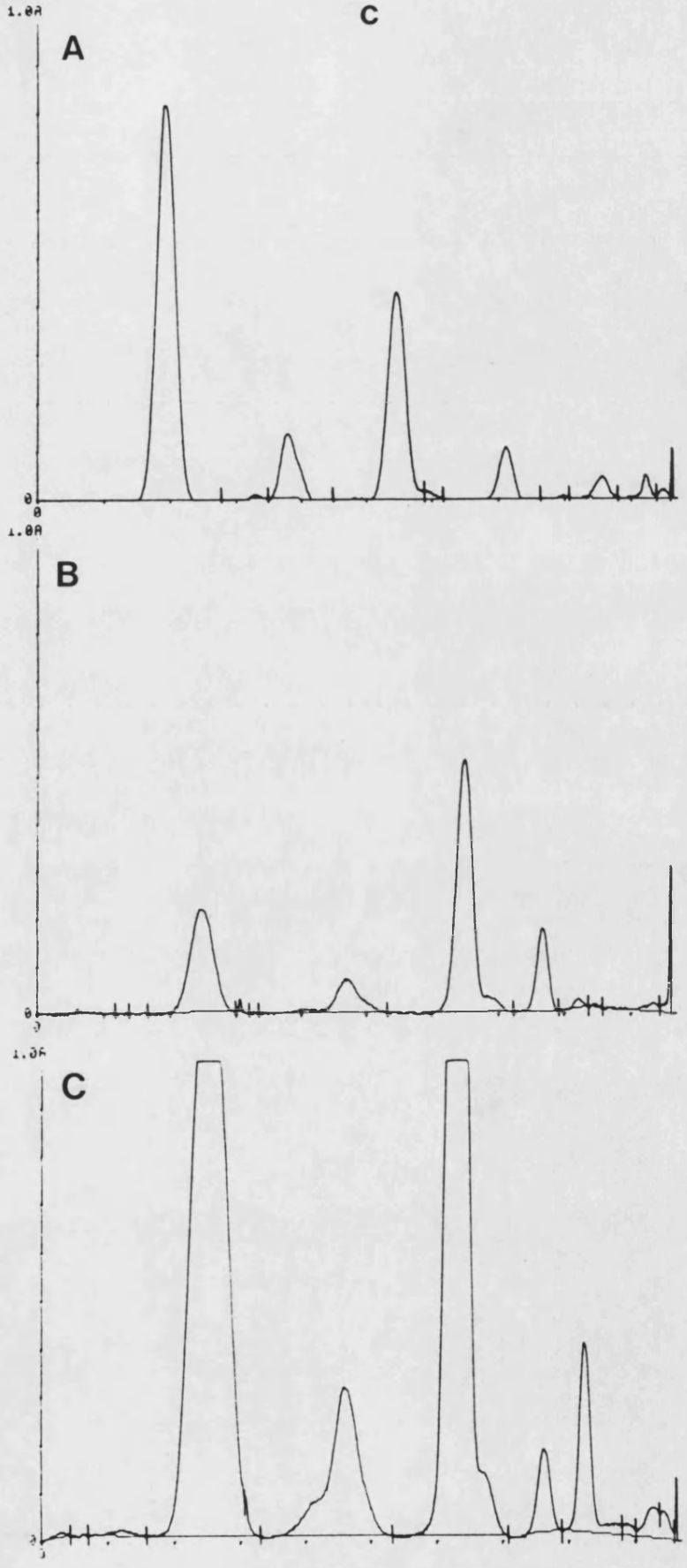
Each diagram represents a different fraction: a) standard parent compound, b) hexane, c) methylene chloride and d) aqueous fractions.

a

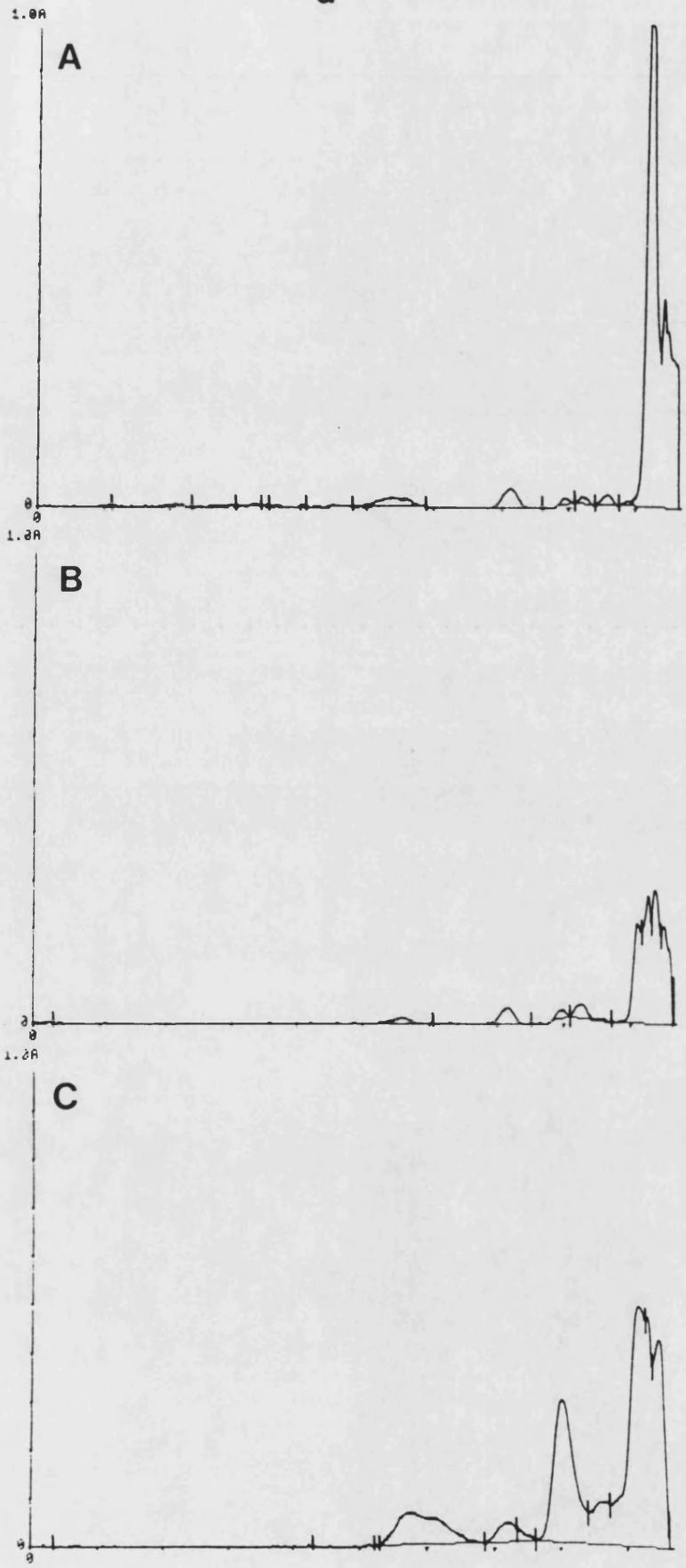


b




C
A
B
C

d



8.4 DISCUSSION

i) Herbicide degradation reactions are generally catalysed by specific enzymes, and their absence in certain species may account for plant susceptibility. Ryan (1981) found that as plant sensitivity increased, there was a parallel decrease in the total quantity of degradation products of isoproturon. In the present investigation, densitometer scanning of autoradiographs of TLC plates indicated the location and density of radioactive spots, which were considered to represent degradation products of isoproturon. Increased numbers of images on plates spotted with barley extracts suggested that this species produced a greater range of metabolites than the *Bromus* spp. This implies that different pathways of degradation may be involved. The discovery of two distinct detoxification pathways in the related chlortoluron, one of which played a greater role in the metabolic reactions in wheat than in grass weeds (Ryan *et al.*, 1981), indicated that the specificity of these reactions was species-dependent. Ryan *et al.* (1981) also showed that chlortoluron was actively hydroxylated *in vivo* by wheat, but minimally by perennial ryegrass. However, the possession of a different metabolic process does not always confer tolerance. For example, Sweetser *et al.* (1982) proposed that an additional pathway of chlorsulfuron degradation in corn caused the crop to be more susceptible, because the resulting product retained considerable herbicidal activity.

In the present study, R_{ip} values were calculated for each radioactive spot, though it was impossible to identify individual metabolites due to an inability to obtain a supply of known standards. For this reason, it was not possible to establish whether

any one metabolite was produced in greater quantities, a feature that has conferred tolerance to other species. In wheat, the *bis*-demethylated metabolite was formed in greater quantities and was less phytotoxic than the mono-demethylated metabolite of isoproturon (Ryan *et al.*, 1981). The densitometer scans presented in this study show the relative intensity of radioactivity associated with each metabolite, thus giving an approximate indication of the amount of each present. A more accurate technique involves removal of the radioactive spots by scraping the chromatograph surface, and subsequent radioassay by liquid scintillation counting. This would have been a worthwhile assay had the metabolites been identified.

Both TLC autoradiography and liquid scintillation counting techniques can only realistically represent degradation if the product of every reaction contains the radiolabelled part of the compound. ^{14}C-isoproturon is labelled on specific carbon atoms (in this case the isopropyl group), however it is known that detoxification of the related urea, chlortoluron, when labelled on the corresponding carbonyl group, produces metabolites all possessing the radioisotope. The metabolic status was expected to be normal at the low concentration of isoproturon used. An experimental period of seven days was assumed to be sufficient for the identification of metabolites in the *Bromus* species, since recovery from photosynthetic inhibition at low concentrations was complete by the fifth day after application of isoproturon (Section 7). Nagi Reddy (1979) observed greater formation of conjugates of isoproturon in wheat than in blackgrass within seven days. His metabolism studies also indicated a relatively rapid degradation capacity in the crop. Rates of degradation were not ascertained for the species

concerned here, and although Müller and Sanad (1975) found no varietal differences in the rate of isoproturon degradation in wheat, there could exist differences between species. Ryan and Owen (1983) reported that 'Maris Otter' barley had an intermediate response to chlortoluron from a range of cultivars tested, thus there is a possibility that isoproturon shows differences in varietal susceptibility in other species. If materials had not been limiting, degradation of ^{14}C-isoproturon would have been investigated over regular intervals, which would have shown whether degradation rates differ between species. In Ryan's work (1981), Maris Otter produced more derivatives of ring-methyl oxidation than N-demethylation of chlortoluron, although it showed neither extreme tolerance or sensitivity. For this reason, it was an ideal choice of cultivar for metabolism studies.

Therefore, it would appear that barley plants possess a mechanism capable of detoxifying isoproturon more effectively than the *Bromus* spp., and this would account for the tolerance shown by barley plants to isoproturon.

9. GENERAL DISCUSSION

This investigation was undertaken with the intention of determining the factors which account for the selectivity of isoproturon to *B.sterilis*, *B. willdenowii* and barley. It was envisaged that selectivity could result from gross morphological differences connected with the availability of the herbicide to either the root or foliar surface, to differential absorption, translocation and inactivation in the tissues of the plant, and finally to differences in susceptibility at the chloroplast level. The conclusion drawn from this study is that no single factor alone can confer tolerance or susceptibility to this herbicide, but that a combination of these probably explains the differential responses of these three species to isoproturon. As Fedtke (1982) pointed out, the capacity for detoxification in itself is not sufficient for the determination of sensitivity or tolerance, but should be seen together with the rates of uptake and translocation. The interdependence of these factors will be emphasized in the following conclusions.

The results of herbicide applications to soil-grown plants suggested that *B. sterilis* was the most susceptible of the three species to isoproturon. Seed depth experiments indicated that *B. sterilis* was more vulnerable in the soil, possibly associated with its smaller, shallower rooting system and the position of the growing point in relation to the herbicide in the soil profile. It would appear that soil uptake was more important than foliar uptake, since dye retention (and hence probably herbicide retention) by this species was poor. The long trichomes on the laminae of *B. sterilis* may prevent access of the spray solution. A

lack of basipetal mobility may contribute to the relatively poor performance of foliar-applied isoproturon. Uptake via the roots was efficient in this species, suggesting that penetration, movement across the root tissue, and xylem loading was rapid. ^{14}C-isoproturon uptake and long distance movement was also extremely rapid in comparison with rates in barley seedlings. Metabolism studies indicated that *B. sterilis* produced fewer metabolites of ^{14}C-isoproturon than *B. willdenowii* or barley plants. Slow recovery from inhibition of carbon dioxide fixation suggested that *B. sterilis* plants were capable of detoxifying isoproturon, but that the rates of uptake and translocation exceeded the rate of detoxification. Rapid translocation ensured thorough distribution of isoproturon throughout the plant, as indicated by a reduction in photosynthesis. Since experiments with isolated chloroplasts showed that the amount of isoproturon required to inhibit photosynthetic electron transport was similar in *B. sterilis* and barley, susceptibility at the chloroplast level cannot be responsible for the sensitivity of this species. Therefore, a combination of morphological features, rapid uptake and translocation and poor detoxification are likely to render *B. sterilis* more susceptible to isoproturon.

By contrast, *B. willdenowii* was poorly controlled in pot trials, although its seed was shown to be the most sensitive to isoproturon in germination tests. Retention of spray droplets on leaf surfaces was relatively high, in spite of the fact that trichomes were present. Uptake of ^{14}C-isoproturon from nutrient solution was rapid and the fresh weight of *B. willdenowii* plants was greatly reduced. However, acropetal movement of the herbicide was extremely slow, suggesting either that xylem loading was slow or

that unloading into cytoplasm was occurring relatively rapidly. Both of these possibilities suggest that a large amount of isoproturon could reach the chloroplast, unless the compound is immobilised in the tissues. The I_{50} value for *B. willdenowii* indicated that slightly greater quantities of isoproturon were required to inhibit photosynthesis in this species, nevertheless, carbon dioxide fixation rates were reduced considerably by relatively low concentrations. The rate of recovery from photosynthetic inhibition indicated that detoxification of isoproturon in *B. willdenowii* was slow, although thin-layer chromatography experiments indicated the production of a number of ^{14}C-isoproturon metabolites. It could be concluded that efficient detoxification, coupled with poor mobility conferred tolerance to this species.

Barley plants generally showed the greatest degree of tolerance to isoproturon in pot trials. This was probably a result of both physical and biochemical factors, the former relating to greater seed vigour, a less vulnerable position of the growing point in soil, and a relatively larger seedling size. Retention on leaf surfaces was greater than on the densely hairy *B. sterilis* although not as great as on *B. willdenowii* seedlings. Uptake of ^{14}C-isoproturon from nutrient culture was extremely slow in comparison with the rate of movement into *Bromus* spp. Translocation of this compound through the leaf was less rapid than in *B. sterilis*. This may have been associated with a more efficient detoxification of isoproturon in barley, as indicated by the fast recovery from photosynthetic inhibition. The presence of more metabolites on TLC autoradiographs of extracted barley plants also indicate that barley detoxifies isoproturon before it accumulates in harmful quantities at the thylakoid membrane.

This work has shown that it is important to be aware of the technical limitations of the individual experimental approach when investigating selectivity. For example, it must be assumed that isolated components of a system function in the same way independently as when they are present as a whole unit, although this is almost certainly not the case. Careful interpretation of the data is required where more than one agent may be responsible for a particular result; for example, the reduction in plant fresh weight following application of isoproturon to the soil may be attributed to many different factors including herbicide availability, uptake, translocation and detoxification. It is obviously necessary to examine these aspects in isolation using *in vitro* assays, although there is a danger of straying too far from the *in vivo* situation in the field. Problems have been identified when using model systems, for example, Geissbühler *et al.* (1963a) noticed that differential absorption in two plant species can be the reverse in nutrient solution when compared with soil. Greater reliability of results will be obtained from continuous, non-destructive techniques (where the same plant material can be reused) such as chlorophyll fluorescence, than from in this case destructive methods such as using the IRGA. When using the latter, standardisation of materials is of paramount importance, and plants should be chosen for uniformity of size.

There are many instances where standardisation of materials and conditions is important. The need to standardise plant age is emphasised by the results of experiments that were designed to investigate the effect of stage of growth on plants at treatment. Even at the early age of 14 days old, plants varied in size, but effects on susceptibility were expected to be minimal. Only with large variations in size have differences in retention (Verity

et al., 1981), and absorption and translocation (Yamaguchi and Crafts, 1958) of herbicides been identified.

The effects of climatic conditions were outside the scope of the present study, although they are likely to be important in the field. By standardising the environmental conditions in a growth cabinet, it was hoped to eliminate the influence of such factors as temperature, relative humidity and light intensity. It is not possible to simulate exactly the fluctuating field conditions, but some attempt was made to maintain similar conditions to those found in the field at the time of seed germination and seedling growth.

During this study, certain potential problems of the experimental techniques employed have been identified. Firstly, following isoproturon application to soil, the distribution and quantity of the active ingredient is based on deductions from the leaching data of other workers. It would be useful to know the precise location of the herbicide in the soil profile, its availability to the plant surface, and the relative amounts absorbed by plants. This type of study would require large quantities of radiolabelled herbicide, but because of a severe shortage of ^{14}C-isoproturon, experiments using the isotope were limited to a brief investigation of uptake and translocation by the three species from nutrient solution.

Secondly, retention on leaf surfaces following spraying with a water-soluble dye does not necessarily simulate retention of a particular herbicide. It would have been valuable to determine the exact quantity of ^{14}C-isoproturon retained, and further to examine the effects of droplet sizes, volume rates and the inclusion of surfactants or additives to the spray. There are several reports of new recommendations to improve the performance of isoproturon in

cereals using additives, for example, in 1985, Chiltern Farm Chemicals/Agrisearch recommended the use of Cropspray 11E for the improved control of blackgrass (Anon, 1985). Thirdly, as far as the biochemical investigations are concerned, they offer a reasonable representation of the mode of action of isoproturon, although this work could be extended to examine the proposal by Pfister *et al.* (1979) that the inhibitory activity of a herbicide depends on its ability to specifically bind to chloroplast membranes. Binding studies similar to those of Dexter *et al.* (1971) would have been valuable. Lastly, the pathways and products of herbicide metabolism require more detailed study. A more precise method of quantifying the metabolites by liquid scintillation, and product identification using known standards would further elucidate the contribution of herbicide detoxification to selectivity.

Since the underlying purpose of this study was to outline the conditions best suited to the control of *B. sterilis* in the field, a summary of these requirements will conclude this thesis.

Where cultural control is still practised, ploughing to invert the topsoil, thus exposing buried seeds, followed by herbicide application will reduce seedling numbers. Where minimal cultivation is preferred, selective spraying at the 1st - 2nd leaf stage of the weed will ensure maximum control. Good cover of the leaves and plant bases must be achieved, since basipetal movement of this compound is absent. Spraying prior to, or following light rainfall will make the herbicide more mobile in the soil profile, and increase phytotoxic activity. If possible, spraying should be delayed until the crop has passed the 3 leaf stage, despite the relative tolerance of young barley plants to isoproturon. Because shallow-sown crops may be especially prone to herbicide damage, drilling to 2 - 3 cm is recommended.

The results presented in this thesis explain much of the reported unreliability of this herbicide in the field. There are obviously many factors affecting selectivity, such as the direct or indirect effects of environmental conditions on herbicide activity. The effects of environmental influences on the performance of isoproturon require detailed study before results from controlled-environment experiments can be related to field conditions.

10. REFERENCES

Abei, H. and Ebner, L. (1962). The fate of the herbicide N-4(P-chlorophenoxy)-phenyl-N'N',-dimethylurea (CIBA 1983) in soil and plants. *Proc. 6th Br. Weed Control Conf.*, **2**, 479-480.

Aberg, E. and Stecko, V. (1976). Internal factors affecting toxicity. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 2, (ed. Audus, L.J.) 175-203, Academic Press, London.

Addala, M.S.A., Hance, R.J. and Drennan, D.S. (1985). Studies on the site of uptake by plants of chlortoluron and terbutryne. *Weed Res.*, **25**, 151-158.

Alexandre, F. (1983). Is *Bromus catharticus* well utilised by live-stock? *Latest tech. inf. on B. catharticus*, Bureau de promotion de varieties fourrageres, Paris.

Allen, F.C., Hartley, M.J. and Meeklah, F.A. (1974). Control of barley grass by ethofumesate. *Proc. 27th New Zealand Weed and Pest Control Conf.*, 85-89.

Anon (1977). *"Technical Bull. on Isoproturon"*, Rhone-Poulenc Agrochemicals.

Anon (1979). Herbicide residue levels and redrilling of failed cereals. *MAFF ADAS S.E. Region Ag. Sci. Service Ann. Rep.*, H.M.S.O., London.

Anon (1980). Sterile brome - some hopes of control but no final answer yet. *Farmers Weekly*, (Arable suppl.), **92**, 28-29, 34.

Anon (1981). Barren Brome. *MAFF ADAS Leaflet 777*, H.M.S.O., Edinburgh.

Anon (1984a). New brome grasses for forage. *NIAB Leaflet 04/11 Herbage*, Cambridge.

Anon (1984b). *"The Agrochemicals Handbook"*, RSC, Unwin Bros., U.K.

Anon (1984c) *"MAFF Approved Products for Farmers and Growers"*, 177-178, H.M.S.O., London.

Anon (1985). Cereal trials. *Farmers Weekly*, **103**, (18), 21.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxide in *Beta vulgaris*. *Pl. Physiol.*, **24**, 1-15.

Ashton, F.M. (1965). Relationship between light and toxicity symptoms caused by atrazine and monuron. *Weeds*, **13**, 164-168.

Ashton, F.M., Gifford, E.M. and Bisalputra, T. (1963). Structural changes in *Phaseolus vulgaris* induced by atrazine II. Effects on fine structure of chloroplasts. *Bot. Gaz.*, **124**, 336-343.

Ashton, F.M., De Villiers, O.T., Glenn, R.F. and Duke, W.B. (1977). Localization of metabolic sites of action of herbicides. *Pestic. Biochem. Physiol.*, **7**, 122-141.

Ashworth, R. de B. and Lloyd, G.A. (1961). Laboratory and field tests
for evaluating the efficiency of wetting agents used in agriculture. *J. Sci. Fd. Agric.*, **12**, 234-240.

Atkin, J.C. and Foster, D.K. (1981). Problems and experiences in simultaneously controlling grass and broad-leaved weeds in wheat and barley. In *"Grass Weeds in Cereals in the U.K."*, 329-336, AAB, Wellesbourne.

Ayres, P. and Cussans, G.W. (1980). The influence of volume rate, nozzle size, and forward speed on the activity of three herbicides for the control of weeds in winter cereals. In *"Spraying systems for the 1980s"*, mon. 24 (ed. Walker, J.O.), 57-64, BCPC, Croydon.

Ayres, P. and Richardson, W.G. (1981). Some pot experiments to investigate potential control of *Bromus sterilis* by various herbicides. In *"Grass Weeds in Cereals in the U.K."*, 265-272, AAB, Wellesbourne.

Baker, E.A. (1980). Effect of cuticular components on foliar penetration. *Pestic. Sci.*, **11**, 367-370.

Baker, E.A. and Procopiou, J. (1980). Effect of soil moisture status on leaf surface wax yield of some drought-resistant species. *J. Hort. Sci.*, **55**, 85-87.

Baker, N.R. and Bradbury, M. (1981). Possible applications of chlorophyll fluorescence techniques for studying photosynthesis *in vivo*. In *"Plants and the daylight spectrum"*, (ed. Smith, H.), Academic Press, New York, 366-373.

Barloy, J. (1982). Possible place of *B. carthartícus* in the forage systems of western France. *Latest tech. inf. on B.catharticus*, Paris.

Bartley, M.R. and Frankland, B. (1984). Phytochrome intermediates and action spectra for light perception by dry seeds. *Pl. Physiol.*, **74**, 601-604.

Betin, M. and Mansat, P. (1979). Agronomic value of various *Bromus* species in France. *Biuletyn Instytutu Hodowli i Aklimatyzacji Róslin*, **135**, 102-123.

Betteridge, K. and Baker, C.J. (1983). Production from a drought-prone Northland pasture direct drilled with 3 grass cultivars. *New Zealand J. Exp. Agric.*, **11**, 101-106.

Bingham, S.W. and Shaver, R. (1971). Uptake, translocation and degradation of diphenamid in plants. *Weed Sci.*, **19**, 639-643.

Black, I.A. and Hewson, R.T. (1978). Isoproturon - a broad-spectrum approach to weed control in winter cereals. *Proc. Br. Crop Protection Conf. - Weeds*, 3, 875-881, BCPC, Croydon.

Black, I.A. and Hewson, R.T. (1982). Weed control with ioxynil plus bromoxynil alone and in tank-mixtures with other herbicides applied in the autumn/winter. In *"Broad-leaved weeds and their control in cereals"*, AAB, Wellesbourne.

Blackman, G.E., Holly, K. and Roberts, H.A. (1949). The comparative toxicity of phytocidal substances. *Symp. Soc. Exp. Biol.*, 3, 283-317.

Blackman, G.E. and Roberts, H.A. (1950). Studies in selective weed control. II. The control of annual weeds in spring cereals. *J. Agric. Sci.*, 40, 70-81.

Blackman, G.E., Bruce, R.S. and Holly, K. (1958). Studies in the principles of phytotoxicity v. interrelationships between specific differences in spray retention and selective toxicity. *J. Exp. Bot.*, 9, 175-205.

Blair, A.M. (1978). Some studies on the sites of uptake of chlortoluron, isoproturon and metoxuron by wheat, *Avena fatua* and *Alopecurus myosuroides*. *Weed Res.*, 18, 381-387.

Blair, A.M. (1985). Influence of soil moisture on isoproturon activity against *Alopecurus myosuroides*. *Weed Res.*, 25, 141-149.

Blair, A.M., Richardson, W.G. and West, T.M. (1983). The influence of climatic factors on metoxuron activity on *Bromus sterilis* L. *Weed Res.*, 23, 259-265.

Börner, H. (1964). Unterschungen mit C^{14}-Mariertem afalon(3,4-dichlorophenyl-metroxymethylharnstoff). *Z. Pflanzenkrankh. Sonder.*, 2, 41-42.

Brian, R.C. (1976). The history and classification of herbicides. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 1, (ed. Audus, L.J.), 1-50, Academic Press, London.

Bucha, H.C. and Todd, C.W. (1951). 3-(p-Chlorophenyl)-1,1-Dimethyl-urea - a new herbicide. *Science*, **114**, 493-494.

Budd, E.G. (1981). Survey, dormancy and life cycle of *Bromus sterilis* (sterile brome) in cereals, with particular reference to spring barley. In *"Grass Weeds in Cereals in the U.K."*, 23-29, AAB, Wellesbourne.

Bukovac, M.J. (1976). Herbicide entry into plants. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 1, (ed. Audus, L.J.), 335-360, Academic Press, London.

Cadahia, E., Ducruet, J.M. and Gaillardon, P. (1982). Whole leaf fluorescence as a quantitative probe of detoxification of the herbicide chlortoluron in wheat. *Chemosphere*, **11**, 445-450.

Challen, S.B. (1962). The retention of aqueous suspensions on leaf surfaces. *J. Pharm. Pharmcol.*, **14**, 707-714.

Chancellor, R.J. and Froud-Williams, R.J. (1984). A second survey of cereal weeds in central southern England. *Weed Res.*, **24**, 29-36.

Chenault, E.W. and Wiese, A.F. (1977). Annual grass control in irrigated alfalfa. *Proc. 30th Southern Weed Sci. Soc. Mtg.*, 147-151.

Clark, A. (1984). Studies on the mode of action and basis of selectivity of the herbicide M & B 34552 in *Cyperus rotundus* L. and *Zea mays* L. *Ph.D. Thesis*, University of Strathclyde.

Corbett, J.R., Wright, K. and Baillie, A.C. (1984). *"The Biochemical Mode of Action of Pesticides"*, Academic Press, London.

Cousens, R., Pollard, F. and Denner, R.A.P. (1985). Competition between *Bromus sterilis* and winter cereals. *Aspects of Applied Biology*, **9**, 67-74, AAB, Wellesbourne.

Crafts, A.S. (1956). Weed control: Applied botany. *Amer. J. Bot.*, **43**, 548-556.

Crafts, A.S. (1959). Further studies on comparative mobility of labelled herbicides. *Pl. Physiol.*, **34**, 613-620.

Crafts, A.S. and Foy, C.L. (1962). The chemical and physical nature of plant surfaces in relation to the use of pesticides and to their residues. *Residue Rev.*, **1**, 112-139.

Critchley, C. and Smillie, R.M. (1981). Leaf chlorophyll fluorescence as an indicator of high light stress (photoinhibition) in *Cucumis sativus* L. *Aust. J. Pl. Physiol.*, **8**, 133-141.

Crosby, D.G. (1976). Nonbiological degradation of herbicides in the soil. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 2, (ed. Audus L.J.), 65-98, Academic Press, London.

Culleton, N. and McCarthy, V. (1983). Germination of *Lolium perenne*, *Bromus willdenowii* and *Trifolium pratense*. *Irish J. Agric. Res.*, 22, 31-36.

Currier, H.B. and Dybing, C.D. (1959). Foliar penetration of herbicides - review and present status. *Weeds*, **7**, 195-213.

Cussans, G.W., Moss, S.R., Hance, R.J., Embling, S.J., Caverly, D.J., Marks, T.G. and Palmer, J.J. (1982). The effect of tillage method & soil factors on the performance of chlortoluron and isoproturon. *Proc. Br. Crop Protection Conf. - Weeds*, **1**, 153-160, BCPC, Croydon.

Cutting, O. (1980). Two spray programme controls sterile brome. *Arable Farming*, 7, no. 9, 30, 33.

Davies, P.J., Drennan, D.S.H., Fryer, J.D. and Holly, K. (1967). The basis of the differential phytotoxicity of 4-hydroxy-3,5-di-iodobenzonitrile. I. The influence of spray retention and plant morphology. *Weed Res.*, **7**, 220-233.

Deloraine, J., Desaymard, P., Desmoras, J., Dodel, J.B., Guillot, M. Jacquet, P., Metivier, R.J. and Planard, A. (1965). A new pre- and post-emergence herbicide - 3-(2,4-dichlorophenyl)-5-tert. butyl-1,3,4-oxidiazon-2-one. *Proc. EWRS Symp. - New Herbicides*, 85-97, Paris.

Dexter, A.G., Slife, F.W. and Butler, H.S. (1971). Detoxification of 2,4-D by several plant species. *Weed Sci.*, **19**, 721-726.

Dicks, J.W. (1978). Inhibition of the Hill reaction of isolated chloroplasts by herbicidal phenylureas. *Pestic. Sci.*, **9**, 59-62.

Dodge, A.D. (1982). The role of light and oxygen in the action of photosynthetic inhibitor herbicides. In *"Biochemical Responses Induced by Herbicides"*, (ed. Moreland, D.E., St. John, J.B. and Hess, F.D.), 57-77, American Chemical Society, Washington D.C.

Dodge, A.D. (1983a). Toxic oxygen species and herbicide action. In *"Pesticide Chemistry - Human Welfare and the Environment"*, vol. 3, (ed. Miyamoto, J. and Kearney, P.C.), 59-66, Pergamon, Oxford.

Dodge, A.D. (1983b). The mode of action of herbicides. In *"Progress in Pesticide Biochemistry and Toxicology"*, vol. 3, (ed. Hutson, D.H. and Roberts, T.R.), 163-197, John Wiley, Chichester.

Duysens, L.N.M. (1964). Photosynthesis. *Progr. Biophys. Mol. Biol.*, **14**, 1-104.

Duysens, L.N.M. and Sweers, H.E. (1963). In *"Microalgae and Photosynthetic Bacteria"*, (Jap. Soc. Pl. Physiol.), 353-372, Univ. Tokyo Press, Tokyo.

Dybing, C.D. and Currier, H.B. (1961). Foliar penetration by chemicals. *Pl. Physiol.*, **36**, 169-174.

Elliott, J.G., Church, B.M., Harvey, J.J., Holroyd, J., Hulls, R.H. and Waterson, H.A. (1979). Survey of the presence and methods of control of wild oat, blackgrass and couch grass in cereal crops in the U.K. during 1977. *J. Agric. Sci.*, **92**, 617-634.

Emami Saravi, R. (1979). Studies on the varietal susceptibility in winter wheat to substituted phenylurea herbicides. *Ph.D. Thesis*, University of London.

Eshel, Y. and Prendeville, G.N. (1967). A technique for studying root vs. shoot uptake of soil-applied herbicides. *Weed Res.*, **7**, 242-245.

Etève, A. (1982). The main characteristics of *Bromus catharticus*. *Latest tech. inf. on B. carthartícus*, Paris.

Fedtke, C. (1973). Effects of the herbicide methabenzthiazuron on the physiology of wheat plants. *Pestic. Sci.*, **4**, 653-664.

Fedtke, C. (1974). Influence of methabenzthiazuron on ATP-level and protein synthesis in wheat. *Pestic. Biochem. Physiol.*, **4**, 386-392.

Fedtke, C. (1982). *"Biochemistry and Physiology of Herbicide Action"*, Springer-Verlag, Berlin.

Fedtke, C., Deichgräber, G. and Schnepf, E. (1977). Herbicide induced changes in wheat chloroplast ultrastructure and chlorophyll a/b ratio. *Biochem. Physiol. Pflanzen*, **171**, 307-312.

Fedtke, C. and Schmidt, R.R. (1983). Behaviour of metribuzin in tolerant and susceptible soybean varieties. In *"Pesticide Chemistry - Human Welfare and the Environment"*, vol. 3, (ed. Miyamoto, J. and Kearney, P.C.), 177-182, Pergamon, Oxford.

Fletcher, W.W. and Kirkwood, R.C. (1982). *"Herbicides and Plant Growth Regulators"*, Granada, London.

Floyd, R.A., Chance, B. and Devault, D. (1971). Low temperature photo-induced reactions in green leaves and chloroplasts. *Biochim. Biophys. Acta.*, **226**, 103-112.

Fournier, J -C., Soulas, G., Catroux, G. and Salle, J. (1975). Degradation microbienne de l'isoproturon dans des modeles de laboratoire. *Chemosphere*, **4**, 207-214.

Fournier, J -C., Massenot, D. and Drogoul, C. (1981). Effect of various pesticides on the degradation of 2,4-D, isoproturon and propamocarb. Importance of some experimental conditions. *Proc. EWRS Symp. - Theory and Practice of the use of Soil Applied Herbicides*, 103-110, Paris.

Franke, W. (1967). Mechanisms of foliar penetration of solutions. *Ann. Rev. Pl. Physiol.*, **18**, 281-300.

Fraser, T.J. (1982). Evaluation of 'Grasslands Matua' prairie grass and 'Grasslands Maru' phalaris with and without lucerne in Canterbury. *New Zealand J. Exp. Agric.*, **10**, 235-237.

Friend, D.J.C. (1961). The control of chlorophyll accumulation in leaves of Marquis wheat by temperature and light intensity. *Physiol. Plant.*, **14**, 28-39.

Froud-Williams, R.J. (1981). Germination behaviour of *Bromus* species and *Alopecurus myosuroides*. In *"Grass Weeds in Cereals in the U.K."*, 31-40, AAB, Wellesbourne.

Froud-Williams, R.J. (1982). Barren brome is on the move. *Farmers Weekly*, **96**, (3), vi-vii.

Froud-Williams, R.J. (1983). The influence of straw disposal and cultivation regime on the population dynamics of *Bromus sterilis*. *Ann. Appl. Biol.*, **103**, 139-148.

Froud-Williams, R.J., Pollard, F. and Richardson, W.G. (1980). Barren brome: a threat to winter cereals? *AFRC WRO 8th biennial report*, 43-51.

Froud-Williams, R.J., Chancellor, R.J. and Drennan, D.S.H. (1981). Potential changes in weed floras associated with reduced-cultivation systems for cereal production in temperate regions. *Weed Res.*, **21**, 99-109.

Froud-Williams, R.J. and Chancellor, R.J. (1982). A survey of grass weeds in cereals in central southern England. *Weed Res.*, **22**, 163-171.

Froud-Williams, R.J., Drennan, D.S.H. and Chancellor, R.J. (1984). The influence of burial and dry-storage upon cyclic changes in dormancy, germination and response to light in seeds of various arable weeds. *New Phytol.*, **96**, 473-481.

Fryer, J.D. and Chancellor, R.J. (1970). Evidence of changing weed populations in arable land. *Proc. 10th Br. Weed Control Conf.*, **3**, 958-964, BCPC, Croydon.

Fryer, J.D. and Makepeace, R.J. (1978). *"Weed Control Handbook: Recommendations"*, British Crop Protection Council vol. II Blackwell, Oxford.

Furmidge, C.G.L. and Osgerby, J.M. (1967). Persistence of herbicides in soil. *J. Sci. Fd. Agric.*, **18**, 269-273.

Geissbühler, H., Haselbach, C., Aebi, H. and Ebner, L. (1963a). The fate of N'-(4-chlorophenoxy)-phenyl-N N-dimethylurea (C-1983) in soils and plants. II. Uptake and distribution within plants. *Weed Res.*, **3**, 181-194.

Geissbühler, H., Haselbach, C., Aebi, H. and Ebner, L. (1963b). The fate of N'-(4-chlorophenoxy)-phenyl-N N-dimethylurea (C-1983) in soils and plants. III. Breakdown in soils and plants. *Weed Res.*, 3, 277-297.

Geissbühler, H. and Voss, G. (1971). In *"Pesticide Terminal Residues"*, (ed. Tahori, A.S.), 305-322, (Suppl. *Pure Appl. Chem.)*, Butterworths, London.

Geissbühler, H., Martin, H. and Voss, G. (1975). The substituted ureas. In *"Herbicides - Chemistry, Degradation and Mode of Action"*, vol. 1, (ed. Kearney, P.C. and Kaufman, D.D.), 209-291, Marcel Dekker Inc., New York.

Gerber, H.R. and Guth, J.A. (1973). Short theory, techniques and practical importance of leaching and adsorption studies. *Proc. EWRS Symp. - Herbicides and the Soil*, 51-69.

Gerber, H.R., Nyffeler, A. and Green, D.H. (1983). The influence of rainfall, temperature, humidity and light on soil - and foliage-applied herbicides. In *"Aspects of Applied Biology"*, 4, 1-14, AAB, Wellesbourne.

Giffard, S.C., Putwain, P.D., Collin, H.A. and Hewett, R.H. (1983). Varietal tolerance of barley to asulam. In *"Aspects of Applied Biology"*, 3, 73-82, AAB, Wellesbourne.

Govindjee, and Papageorgiou, G. (1971). Chlorophyll fluorescence and photosynthesis: fluorescence transients. In *"Photophysiology"*, vol. 6, (ed. Giese, A.C.), 1-46, Academic Press, New York.

Gray, G.R. (1981). Aspects of the ecology of barren brome *(Bromus sterilis* L.). *D. Phil. Thesis*, University of Oxford.

Gray, R.A. and Weierich, A.J. (1969). Importance of root, shoot and seed exposure on the herbicidal activity of EPTC. *Weed Sci.*, 17, 223-229.

Greene, D.W. and Bukovac, M.J. (1974). Stomatal penetration: effect of surfactants and role in foliar absorption. *Amer. J. Bot.*, **61**, 100-106.

Gressel, J. (1982). Triazine herbicide interaction with a 32,000 M_r thylakoid protein - alternative possibilities. *Plant Sci. Lett.*, **25**, 99-106.

Grime, J.P. (1979). *"Plant Strategies and Vegetation Processes"*, John Wiley, Chichester.

Grime, J.P. and Hunt, R. (1975). Relative growth-rate : its range and adaptive significance in a local flora. *J. Ecol.*, **63**, 393-422.

Grime, J.P. and Jarvis, B.C. (1975). Shade avoidance and shade tolerance in flowering plants. In *"Light as an ecological factor"*, (ed. Bainbridge, R., Evans, G.C. and Rackman, D.), 525-532, Blackwell, Oxford.

Gross, D., Laanio, T., Dupuis, G. and Esser, H.O. (1979). The metabolic behaviour of chlortoluron in wheat and soil. *Pestic. Biochem. Physiol.*, **10**, 49-59.

Grover, R. (1966). Influence of organic matter, texture and available water on the toxicity of simazine in soil. *Weeds*, **14**, 148-151.

Grover, R. (1970). Influence of soil-moisture content on the bio-activity of picloram. *Weed Sci.*, **18**, 110-111.

Hagimoto, H. and Yoshikawa, H. (1972). Synergistic interactions between inhibitors of growth and photosynthesis. *Weed Res.*, **12**, 21-30.

Hammerton, J.L. (1966). Studies on weed species of the genus *Polygonum* L.III. Variation in susceptibility to 2-(2,4-dichlorophenoxy) propionic acid within *P. lapathifolium*. *Weed Res.*, **6**, 132-141.

Hammerton, J.L. (1967). Environmental factors and susceptibility to herbicides. *Weeds*, **15**, 330-336.

Hammerton, J.L. (1968). Past and future changes in weed species and weed floras. *Proc. 9th Br. Weed Control Conf.*, **3**, 1136-1146, BCPC, Croydon.

Hance, R.J. and McKone, C.E. (1976). The determination of herbicides. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 2, (ed. Audus, L.J.), 393-446, Academic Press, London.

Harper, J.L. (1959). The ecological significance of dormancy. *Proc. 4th Int. Cong. Crop Protection*, 415-420, Hamburg.

Hartley, G.S. (1976). Physical behaviour in the soil. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 2, (ed. Audus, L.J.),1-28, Academic Press, London.

Hartley, G.S. and Graham-Bryce, I.J. (1980). *"Physical Principles of Pesticide Behaviour"*, vol. 2, Academic Press, London.

Hatzios, K.K., Penner, D. and Bell, D. (1979). Inhibition of photosynthetic electron transport in isolated spinach chloroplasts by two 1,3,4-thiadiazolyl derivatives. *Pl. Physiol. (Suppl.)*, **63**, 41.

Hatzios, K.K. and Penner, D. (1985). *"Metabolism of Herbicides in Higher Plants"*, Burgess Publishing Co., Minneapolis.

Hay, J.R. (1976). Herbicide transport in plants. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 1, (ed. Audus, L.J.), 365-396, Academic Press, London.

Hayes, M.H.B. (1970). Adsorption of triazine herbicides on soil organic matter, including a short review on soil organic matter chemistry. *Residue Rev.*, **32**, 131-174.

Hazard, W.H. (1967). Chemical weed control in onions. *Qd. Agric.J.*, **93**, 86-93.

Henderson, J.D. and Grant, D.A. (1974). Effects of oversowing to control barley grass. *New Zealand J. Exp. Agric.*, **2**, 425-427.

Hewitt, E.J. (1966). Sand and water culture methods used in the study of plant nutrition. 2nd revised edition *Commonwealth Bureau Hort. Plant. Crops Tech. Comm.*, no. 22.

Hewson, R.T. (1974). Isoproturon, a new selective herbicide for control of *Alopecurus myosuroides* in winter cereals. *Proc. 12th Br. Weed Control Conf.*, **1**, 75-82, BCPC, Croydon.

Hewson, R.T. and Read, M.A. (1985). Control of meadow-grasses and broad-leaved weeds in cereals with post-emergence isoproturon mixtures. In *"Aspects of Applied Biology"*, **9**, 245-254, AAB, Wellesbourne.

Hibbitt, C.J. (1969). Growth and spray retention of wild oat and flax in relation to herbicidal selectivity. *Weed Res.*, **9**, 95-107.

Hill, R. and Bendall, F. (1960). Function of the two cytochrome components in chloroplasts: a working hypothesis. *Nature*, **186**, 136-137.

Hilton, J.L., Jansen, L.L. and Hull, H.M. (1963). Mechanisms of herbicide action. *Ann. Rev. Pl. Physiol.*, **14**, 353-384.

Hilton, J.R. (1982). An unusual effect of the far-red absorbing form of phytochrome: photoinhibition of seed germination in *Bromus sterilis* L. *Planta*, **155**, 524-528.

Hilton, J.R. (1984). The influence of temperature and moisture status on the photoinhibition of seed germination in *Bromus sterilis* L. by the far-red absorbing form of phytochrome. *New Phytol.*, **97**, 369-374.

Hoffman-Falk, H., Mattoo, A.K., Marder, J.B., Edelman, M. and Ellis, R.J. (1982). *Proc. Natl. Acad. Sci. U.S.A.*, **257**, 4583-4587.

Hogue, E.J. (1978). Absorption and translocation of metobromuron and chlorbromuron. *J. Env. Sci. Health*, **13**, 323-329.

Holly, K. (1956). Penetration of chlorinated phenoxyacetic acid into leaves. *Ann. Appl. Biol.*, **44**, 195-199.

Holly, K. (1976). Selectivity in relation to formulation and application methods. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 2 (ed. Audus, L.J.), 249-278, Academic Press, London.

Holly, K. and Roberts, H.A. (1963). Persistence of phytotoxic residues of triazine herbicides in soil. *Weed Res.*, **3**, 1-10.

Home-Grown Cereals Authority (1980). Planting Survey 1980. *Weekly Bull.*, **14**, 43.

Hubbard, C.E. (1968). *"Grasses"*, The Chaucer Press, Suffolk (Penguin Books.)

Hubbard, K.R., Livingston, D.B. and Ross, B.L. (1976). Chemical control of *Alopecurus myosuroides* in winter cereals. *Proc. Br. Crop Protection Conf. - Weeds*, **1**, 63-70, BCPC, Croydon.

Hulbert, L.C. (1955). Ecological studies of *Bromus tectorum* and other annual brome grasses. *Ecol. Monogr.*, **37**, 181-213.

Hunt, G.M. and Baker, E.A. (1982). Developmental and environmental variations in plant epicuticular waxes: some effects on the penetration of naphthylacetic acid. In *"The Plant Cuticle"*, (ed.

Cutler, D.F., Alvin, K.L. and Price, C.E.), 279-292, Academic Press, Oxford.

Ingram, G.H.and Kyndt, C.F.A. (1981). Isoproturon - the control of *Alopecurus myosuroides* (Blackgrass) - a summary of its performance. In *"Grass Weeds in Cereals in the U.K."*, 231-237, AAB, Wellesbourne.

Jansen, L.L. (1973). Enhancement of herbicides by silicone surfactants. *Weed Sci.*, **21**, 130-135.

Jarvis, R.H. (1981). The integrated effect of herbicides and cultural methods on grass weed control at Boxworth E.H.F. In *"Grass Weeds in Cereals in the U.K."*, 367-376, AAB, Wellesbourne.

Jarvis, R.H. (1982). Brome grass control. *Ann. Rev. Boxworth Experimental Husbandry Farm*, 5-7.

Kaplan, S. and Arntzen, C.J. (1982). In *"Photosynthesis: Energy Conversion by Plants and Bacteria"*, (ed. Govindjee),**1**, 65-150, Academic Press, London.

Kaufman, D.D. and Kearney, P.C. (1976). In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 2, (ed. Audus, L.J.), 29-64, Academic Press, London.

Kautsky, H. and Hirsch, A. (1931). Neue versuche zur kohlenstoff-assimilation. *Naturwissenschaften*, **19**, 964.

Kendrick, R.E. (1976). Photocontrol of seed germination. *Sci. Prog.*, **63**, 347-367.

Kirkwood, R.C. (1972). Leaf surface factors. *Proc. 11th Br. Weed Control Conf.*, **3**, 1117-1128, BCPC, Croydon.

Kirkwood, R.C. (1977). Some criteria determining the efficiency of penetration and translocation of foliage-applied herbicides. In *"Herbicides and Fungicides - Factors affecting their activity"*, (ed. McFarlane, N.R.), 67-80, Alden Press, Oxford.

Kirkwood, R.C., Dalziel, J., Matlib, A. and Somerville, L. (1972). The role of translocation in selectivity of herbicides with reference to MCPA and MCPB. *Pestic. Sci.*, **3**, 307-321.

Knake, E.L., Appleby, A.P. and Furtick, W.R. (1967). Soil incorporation and site of uptake of pre-emergence herbicides. *Weeds*, **15**, 228-232.

Kulshrestha,G. (1983). Persistence of the herbicide isoproturon in soil. In *"Aspects of Applied Biology"*, **4**, 413-422, AAB, Wellesbourne.

Lambert, S.M. (1966). The influence of soil-moisture content on herbicidal response. *Weeds*, **14**, 273-275.

Lavorel, J. and Etienne, A-L. (1971). *In vivo* chlorophyll fluorescence. In *"Primary Processes of Photosynthesis"*, (ed. Barber, J.), 203-268, Elsevier/N. Holland Biomedical Press, Amsterdam.

Leon, J.M. and Bukovac, M.J. (1978). Cuticle development and surface morphology of olive leaves with reference to penetration of foliar-applied chemicals. *J. Amer. Soc. Hort Sci.*, **103**, 465-472.

Leonard, O.A., Bayer, D.E. and Glenn, R.K. (1966). Translocation of herbicides and assimilates in red maple and white ash. *Bot. Gaz.*, **127**, 193-201.

Leonard, O.A., Lider, L.A. and Glenn, R.K. (1966). Absorption and translocation of herbicides by Thompson seedless (Sultanina) grape, *(Vitis vinifera* L.). *Weed Res.*, **6**, 37-49.

Leonard, O.A., Donaldson, T.W. and Bayer, D.E. (1968). Translocation of labelled assimilates into and out of bean leaves as affected by 2,4-D and benzyladenine. *Bot. Gaz.*, **129**, 266-279.

Luscombe, B.M. (1981). Field persistence of an isoproturon/triallate mixture in a granule formation. *Proc. EWRS Symp.-Theory and Practice of the use of Soil Applied Herbicides*, 170-177. Paris.

Luscombe, B.M. (1983). The influence of rainfall and temperature on the persistence of autumn-applied triallate and isoproturon in the 1979/80 and 1980/81 seasons. In *"Aspects of Applied Biology"*, **4**, 423-432, AAB, Wellesbourne.

MacKinney, G. (1941). Absorption of light by chlorophyll solutions. *J. Biol. Chem.*, **140**, 315-322.

Macquarrie, P., Mcleod, J.E., Horton, R. and Stephenson, G.R. (1985). The influence of plant age on tomato tolerance to metribuzin. *Proc. Br. Crop Protection Conf. - Weeds*, **3**, 1179-1185, BCPC, Croydon.

Magenham, G. (1982). As every ~~other~~ crop, *Bromus* has to be treated against weeds. *Latest Tech. inf. on B. catharticus*, Paris.

Martin, J.T. and Juniper, B.E. (1970). *"The Cuticle of Plants"*, Edward Arnold, London.

McAlister, E.D. and Myers, J. (1940). Fluorescence yields and rates of photosynthesis. *Smithsonian Inst. Misc. Collections*, **99**, 1-37.

McIntosh, R.M., Robertson, J. and Kirkwood, R.C. (1981). The mode of action and basis of selectivity of isoproturon in wheat, (wild oats) *Avena fatua* L. and (blackgrass) *Alopecurus myosuroides* L. In *"Grass Weeds in Cereals in the U.K."*, 247-255, AAB, Wellesbourne.

Midgley, S.J. (1982). Effects of surfactants on phenoxyalkanoic herbicides - a preliminary report. *Aspects of Applied Biology*, **1**, 193-200.

Minshall, W.H. (1960). Effect of 3-(4-chlorophenyl)-1,1-dimethyl-urea on dry matter production, transpiration, and root extension. *Can. J. Bot.*, **38**, 201-216.

Minshall, W.H. (1967). *Abstr. Weed Sci. Soc. Amer.*, 67.

Mock, I.T. and Amor, R.L. (1982). Brome grasses (*Bromus spp.*) as contaminants of barley grain in the Victorian Mallee. *Aust. Weeds*, 2, 16-17.

Moreland, D.E. (1974). Interference with ATP generation: Evolution of a hypothesis that accounts for the phytotoxicity of certain herbicides. *Abstr. Weed Sci. Soc. Amer.*, 76.

Moreland, D.E. (1980). Mechanisms of action of herbicides. *Ann. Rev. Pl. Physiol.*, **31**, 597-638.

Moss, S.R. (1979). The influence of tillage and method of straw disposal on the survival and growth of blackgrass, *Alopecurus myosuroides*, and its control by chlortoluron and isoproturon. *Ann. Appl. Biol.*, **91**, 91-100.

Moss, S.R. and Cussans, G.W. (1985). Variability in the susceptibility of *Alopecurus myosuroides* (Blackgrass) to chlortoluron and isoproturon. In *"Aspects of Applied Biology*, **9**, 91-98.

Mudd, P.J., Hance, R.J.and Wright, S.J.L. (1983). The persistence and metabolism of isoproturon in soil. *Weed Res.*, **23**, 239-246.

Müller, V.F. and Sanad, A. (1975). Untersuchungen über die unterschiedliche empfindlichkeit verschiedener weizensorten gegen metoxuron. *Z. Pflkrankh. Sonder.*, 7, 281-291.

Müller, V.F., Frahm, J. and Sanad, A. (1977). Metabolism of phenylurea compounds in wheat varieties with differential herbicide sensitivity. *Mitt. Biol. Bundesanst. Land-Forstwirtsch.*, **178**, 241-242.

Muzik, T.J., Cruzado, H.J. and Loustalot, A.J. (1954). Studies on the absorption, translocation and action of CMU. *Bot. Gaz.*, **116**, 65-73.

Nagi Reddy, A. (1979). The mode of action and basis of selectivity of asulam, isoproturon, MCPA and oxidiazom in selected groups of weed species. *Ph.D. Thesis*, University of Strathclyde.

Naylor, A.W. (1976). Herbicide metabolism in plants. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 1, (ed. Audus, L.J.), 397-428, Academic Press, London.

Nishimoto, R.K. and Warren, G.F. (1971). Shoot zone uptake and translocation of soil-applied herbicides. *Weed Sci.*, **19**, 156-161.

Norris, R.F. and Bukovac, M.J. (1968). Structure of the pear leaf cuticle with special reference to cuticular penetration. *Amer. J. Bot.*, **55**, 975-983.

Okereke, O.U., Blair, A.M. and Caseley, J.C. (1981a). Effects of depth of planting, temperature and soil moisture on seed emergence and survival in soil of *Bromus sterilis* and *Phalaris minor*. In *"Grass Weeds in Cereals in the U.K."*, 41-46, AAB, Wellesbourne.

Okereke, O.U., Blair, A.M. and Caseley, J.C. (1981b). Some factors affecting the activity of isoproturon against *Bromus sterilis* and *Phalaris minor*. *Med. Fac. Landbouwn. Rijksuniv. Gent*, **46**, 83-89.

Orson, J.H. (1981). The control of *Bromus sterilis* in cereals with herbicides - ADAS results 1979 and 1980. In *"Grass Weeds in Cereals in the U.K."*, 291-298, AAB, Wellesbourne.

Pallett, K.E. and Dodge, A.D. (1980). Studies into the action of some photosynthetic inhibitor herbicides. *J. Exp. Bot.*, **31**, 1051-1066.

Palmer, C.G. (1981). Control of *Bromus sterilis* in cereals with cyanazine alone and in mixtures. In *"Grass Weeds in Cereals in the U.K."*, 299-306, AAB, Wellesbourne.

Papageorgiou, G. (1975). Chlorophyll fluorescence: an intrinsic probe of photosynthesis. In *"Bioenergetics of Photosynthesis"*, (ed. Govindjee), 319-371, Academic Press, New York.

Parker, C. (1963). Factors affecting the selectivity of 2,3-dichloroallyl diisopropylthiocarbamate (di-allate) against *Avena* spp. in wheat and barley. *Weed Res.*, **3**, 259-276.

Parker, C. (1966). The importance of shoot entry in the action of herbicides applied to the soil. *Weeds*, **14**, 117-121.

Parneix, P. (1982a). Management of a *Bromus catharticus* crop. *Latest tech. inf. on B. carthartícus*, Paris.

Parneix, P. (1982b). Performance of a new forage species *B. willdenowii* (cv. Bellegarde) in western France. *Sciences Agronomiques*, **2**, 25-36, Rennes.

Penner, D. (1971). Effect of temperature on phytotoxicity and root uptake of several herbicides. *Weed Sci.*, **19**, 571-579.

Percival, M.P. and Dodge, A.D. (1983). Photodynamic effects of rose bengal on senescent flax cotyledons. *J. Exp. Bot.*, **34**, 47-54.

Pestemer, W. (1976). Quantitativer biotest zur bestimmung von photosynthesehemmern im boden. *Weed Res.*, **16**, 357-363.

Peyraud, J.C. (1983). Bientôt un nouveau brome cathartique. *Élevage Bovin*, **130**, 21-23.

Pfister, K. and Arntzen, C.J. (1979). The mode of action of photosystem II specific inhibitors in herbicide resistant weed biotypes. *Z. Naturforsch.*, **34C**, 996-1009.

Pfister, K., Radosevich, S.R. and Arntzen, C.J. (1979). Modification of herbicide binding to photosystem II in two biotypes of *Senecio vulgaris* L. *Pl. Physiol.*, **64**, 995-999.

Pollard, F. (1982). Light induced dormancy in *Bromus sterilis*. *J. Appl. Ecol.*, **19**, 563-568.

Pollard, F. and Richardson, W.G. (1981). Chemical control of *Bromus sterilis* in winter wheat and barley. In *"Grass Weeds in Cereals in the U.K."*, 273-281, AAB, Wellesbourne.

Prendeville, G.N., Eshel, Y., Schreiber, M.M. and Warren, G.F. (1967). Site of uptake of soil-applied herbicides. *Weed Res.*, **7**, 316-322.

Price, C.E. (1973). Uptake and redistribution of N-methyl pyridinium chloride, a model systemic compound, by wheat plants. *Proc. 7th Br. Insecticide and Fungicide Conf.*, 161-169, BCPC, Croydon.

Price, C.E. (1977). Penetration and translocation of herbicides and fungicides in plants. In *"Herbicides and Fungicides - factors affecting their activity"*, (ed. McFarlane, N.R.), 42-66, Alden Press, Oxford.

Priest, B. (1976). The uptake, translocation and metabolism of the herbicide cyanazine in plant seedlings. *Ph.D.Thesis*, University of Bath.

Pusztai, T. and Vegh, A. (1978). Mutations caused by herbicides. *Acta Bot. Acad. Sci. Hung.*, **24**, 327-329.

Rademacher, B. (1959). The control of blackgrass, *Alopecurus myosuroides* by cultivations with the addition of herbicides.

Proc. German Conf. on Weed Biology and Control, 17-21.

Rahman, A. and Ashford, R. (1970). Selective action of trifluralin for control of green foxtail in wheat. *Weed Sci.*, **18**, 754-759.

Redbond, M.R. (1980). Trials with metoxuron for the control of *Bromus sterilis* in winter cereals. *Proc. Br. Crop Protection Conf. - Weeds*, **1**, 275-281, BCPC, Croydon.

Rieder, G., Buchholtz, K.P. and Kust, C.A. (1970). Uptake of herbicides by soybean seed. *Weed Sci.*, **18**, 101-105.

Riely, C.A., Cohen, G. and Lieberman, M. (1974). Ethane evolution: a new index of lipid peroxidation. *Science*, **183**, 208-210.

Renger, G. (1976). Studies on the structural and functional organisation system of photosynthesis. *Biochim. Biophys. Acta*, **440**, 287-300.

Renger, G. (1979). Studies about the mechanism of herbicidal interaction with photosystem II in isolated chloroplasts. *Z. Naturforsch.*, **34C**, 1010-1014.

Richardson, R.G. (1975). Foliar penetration and translocation of 2,4,5-T in blackberry *(Rubus procerus* P.J. Muell.) *Weed Res.*, **15**, 33-38.

Richardson, W.G., Dean, M.L. and Parker, C. (1977). The activity and selectivity of the herbicides: ethofumesate, RU 12709, and isoproturon. *AFRC WRO Tech. Rep.*, **44**.

Richardson, W.G., West, T.M. and Parker, C. (1981). The activity and pre-emergence selectivity of some recently developed herbicides: UBI S-734, SSH-43, ARD 34/02 (=NP55), PP009 and DPX 4189. *AFRC WRO Tech. Rep.*, **62**.

Ridley, S.M. (1977). Interaction of chloroplasts with inhibitors. *Pl. Physiol.*, **59**, 724-732.

Ries, S.K. (1976). Subtoxic effects on plants. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 2, (ed. Audus, L.J.), 313-344, Academic Press, London.

Riley, D. and Morrod, R.S. (1976). Relative importance of factors influencing the activity of herbicides in soils. *Proc. Br. Crop Protection Conf. - Weeds* ,3, 971-980, BCPC, Croydon.

Roberts, H.A. (1982). *"Weed Control Handbook: Principles"*, Blackwell, Oxford.

Robertson, M.M. and Kirkwood, R.C. (1969). The mode of action of foliage-applied, translocated herbicides with particular reference to the phenoxy-acid compounds. I. The mechanism and factors influencing herbicide absorption. *Weed Res.*, **9**, 224-240.

Robertson, M.M. and Kirkwood, R.C. (1970). The mode of action of foliage-applied, translocated herbicides with particular reference to the phenoxy-acid compounds. II. The mechanism and factors influencing translocation, metabolism and biochemical inhibition. *Weed Res.*, **10**, 94-120.

Robertson, M.M., Parham, P.H. and Bukovac, M.J. (1971). Penetration of diphenylacetic acid through enzymatically-isolated tomato fruit cuticle as influenced by substitution on the carboxyl group. *J. Agric. Fd. Chem.*, **19**, 754-757.

Rodriguez, G.E. (1982). Chemical weed control in barley *(Hordeum vulgare* L.). *Weed Abstr.*, **31**, (11), 3390.

Rogers, R.L. and Funderburk, H.H. (1967). Absorption, translocation and metabolism of cotoran by cotton and cucumber. *Abstr. Weed Sci. Soc. Amer.*, 43-44.

Romero, C., Franco, O. and Cárdenas, J. (1969). Chemical control of weeds in wheat and barley in the Bogotá Plain. *Seminar de C.O.M.A.L.F.I.*, 27-28, Bogotá.

Rule, J.S. (1981). Grass weed problems old and new. *Arable Farming*. **8**, no.10, 63, 68.

Rumball, W. (1974). 'Grasslands Matua' prairie grass (*Bromus catharticus* Vahl.) *New Zealand J. Exp. Agric.* **2**, 1-5.

Runyan, T.J. and Peeper, T.F. (1978). Cultural control of *Bromus* species in winter wheat. *Proc. 31st Southern Weed Sci. Soc. Mtg.*, 68.

Ryan, P.J. (1981). Studies on the mechanism of the selective action of chlortoluron in cereals and cereal weeds. *Ph.D. Thesis*. University of London.

Ryan, P.J., Gross, D., Owen, W.J. and Laanio, T.L. (1981). The metabolism of chlortoluron, diuron and CGA 43057 in tolerant and susceptible plants. *Pestic. Biochem. Physiol.*, **16**, 213-221.

Ryan, P.J. and Owen, W.J. (1982). The mechanism of selectivity of chlortoluron between cereals and grass-weeds. *Proc. Br. Crop Protection Conf. - Weeds*, **1**, 317-324.

Ryan, P.J. and Owen, W.J. (1983). Metabolism of chlortoluron in tolerant and sensitive cereal varieties. In *"Aspects of Applied Biology"*, **3**, 63-72, AAB, Wellesbourne.

Sánchez, G. *et al.* (1970). Critical times for applying dinoseb in wheat and oats. *Seminar de C.O.M.A.L.F.I.*, 3-4, Bogotá.

Santakumari, M. and Das, V.S.R. (1978). Herbicidal interference with the photochemical activities of chloroplasts (*in vivo* and *in vitro*) of certain crop and weed species. *Pestic. Biochem. Physiol.*, **9**, 119-129.

Sargent, J.A. (1976). Relationship of selectivity to uptake and movement. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 2, (ed. Audus, L.J.), 303-312, Academic Press, London.

Sargent, J.A. and Blackman, G.E. (1962). Studies on foliar penetration: 1. Factors controlling the entry of 2,4-dichlorophenoxyacetic acid. *J. Exp. Bot.*. **13**, 348-368.

Schulz, H. (1888). *Arch. gen. Physiol.*, **42**, 517-541.

Shone, M.G.T. and Wood,A.V. (1973). *Proc. 7th Br. Insecticide and Fungicide Conf.*, **1**, 151-158, BCPC, Croydon.

Simon, J.C., Le Corre, L. and Coppenet, M. (1983). Essai comparatif de dix graminées fourrâgeres dont six cultivars de bromes dans le finistère. *Fourrages*. **93**, 85-108.

Siriwardana, T.G.D., Blair, A.M. and Bartlett, B.O. (1981). The leaching of chlortoluron, isoproturon and metoxuron as determined by bioassay of soil columns. *Proc. EWRS Symp. - Theory and practice of the use of soil applied herbicides*, 309-317, Paris.

Sitte, P. and Rennier, R. (1963). Untersuchungen an cuticularen zollwandschichten. *Planta. Arch. Wiss. Bot.*, **60**, 19-40.

Smith, J.W. and Sheets, T.J. (1967). Uptake, distribution and metabolism of monuron and diuron by several plants. *J. Agric. Fd. Chem.*, **15**, 577-581.

Standell, C.J. and Haggar, R.J.(1985). The tolerance of *Bromus willdenowii* to various herbicides. *Ann. Appl. Biol.*, **106**, 88-89.

Stanger, C.E. and Appleby, A.P. (1972). A proposed mechanism for diuron-induced phytotoxicity. *Weed Sci.*, **20**, 357-363.

Stickler, R.L., Knake, E.L. and Hinesly, T.D. (1969). Soil moisture and effectiveness of pre-emergence herbicides. *Weed Sci.*, **17**, 257-259.

Strang, R.H. and Rogers, R.L. (1971). A microautoradiographic study of ^{14}C-diuron absorption by cotton. *Weed Sci.*, **19**, 355-369.

Swanson, C.R. and Swanson, H.R. (1968). Metabolic fate of monuron and diuron in isolated leaf discs. *Weed Sci.*, **16**, 137-143.

Sweetser, P.B. and Todd, C.W. (1961). The effect of monuron on oxygen liberation in photosynthesis. *Biochim. Biophys. Acta*, **51**, 504-508.

Sweetser, P.B., Schow, G.S. and Hutchison, J.M. (1982). Metabolism of chlorsulfuron by plants: biological basis for selectivity of a new herbicide for cereals. *Pestic. Biochem. Physiol.*, **17**, 18-23.

Tanton, T.W. and Crowdy, S.H. (1972). Water pathways in higher plants. *J. Exp. Bot.*, **23**, 600-618.

Thizy, A., Pillon, D., Poignant, P. and Rognon, J. (1972). Activité herbicide sélective de la N-(isopropyl-4-phenyl)-N',N'-dimethylurea. *C.R. de L'Academie des Sciences de Paris*, 2053-2056.

Thompson, H.E., Swanson, C.P. and Norman, A.G. (1946). New growth-regulating compounds. 1. Summary of growth-inhibitory activities of some organic compounds as determined by three tests. *Bot. Gaz.*, **107**, 476-507.

Thompson, K. and Grime, J.P. (1979). Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *J. Ecol.*, **67**, 893-921.

Toole, V.K. (1973). Effects of light, temperature and their interactions on the germination of seeds. *Seed Sci. Technol.*, **1**, 339-396.

Tottman, D.R., Holroyd, J., Lupton, F.G.H., Oliver, R.H., Barnes, T.R. and Tysoe, R.H. (1975). The tolerance of chlortoluron and isoproturon by varieties of winter wheat. *Proc. EWRC Symp.- Status and Control of Grassweeds in Europe*, 360-368, Paris.

Trebst, A. (1980). Inhibitors of electron flow: tools for the functional and structural localisation of carriers and energy conservation sites.In *"Methods in Enzymology"*, (ed. San Pietro, A.),**69**, 675, Academic Press, New York.

Trebst, A. and Avron, M. (eds.) (1977). *"Encyclopedia of Plant Physiology"*, **5**, Springer-Verlag, New York.

Trebst, A., Draber, W. and Donner, W.T. (1983). Mode of action and MO calculation of two classes of herbicides interacting with the reducing side of photosystem II. In *"Pesticide Chemistry: Human Welfare and the Environment"*, **3**, (ed. Miyamoto, J. and Kearney, P.C.), 85-90, Pergamon, Oxford.

Upchurch, R.P. (1957). The influence of soil-moisture content on the response of cotton to herbicides. *Weeds*, **5**, 112-120.

Van Assche, C.J. and Carles, P.M. (1981). Photosystem II inhibiting chemicals: molecular interaction between inhibitors and a common target. In *"Biochemical Responses Induced by Herbicides"*, (ed. Moreland, D.E., St. John, J.B. and Hess, F.D.), 1-22, American Chemical Society, Washington D.C.

Van Leeuwen, P.H. and Van Oorschot, J.L.P. (1976). Effects of some phenylurea herbicides on photosynthesis of two wheat varieties. *Weed Res.*, **16**, 11-14.

Van Oorschot, J.L.P. (1965). Selectivity and physiological inactivation of some herbicides inhibiting photosynthesis. *Weed Res.*, **5**, 84-97.

Van Oorschot, J.L.P. (1968). The recovery from inhibition of photosynthesis by root-applied herbicides as an indication of herbicide inactivation. *Proc. 9th Br. Weed Control Conf.*, **2**, 624-632, BCPC, Croydon.

Van Oorschot, J.L.P. (1970). Effect of transpiration rate of bean plants on inhibition of photosynthesis by some root-applied herbicides. *Weed Res.*, **10**, 230-242.

Van Oorschot, J.L.P. (1979). Types of selective action by herbicides which inhibit photosynthesis. *Z. Naturforsch.*, **34C**, (11), 900-904.

Van Overbeek, J. (1956). Absorption and translocation of plant ~~growth~~ regulators. *Ann. Rev. Pl. Physiol.*, **7**, 355-372.

Van Rensen, J.J.S. (1982). Molecular mechanisms of herbicide action near photosystem II. *Physiol. Plant*, **54**, 515-521.

Veerasekaran, P., Kirkwood, R.C. and Fletcher, W.W. (1976). The mode of action of asulam (methyl 4-aminobenzene sulphonyl carbamate) in bracken. *Bot. J. Linn. Soc.*, **73**, 247-268.

Veerasekaran, P., Kirkwood, R.C. and Fletcher, W.W. (1977). Studies on the mode of action of asulam in bracken (*Pteridium aquilinum* L. Kuhn). II. Biochemical activity in the rhizome buds. *Weed Res.*, **17**, 85-92.

Verity, J., Walker, A. and Drennan, D.S.H. (1981). Aspects of the selective phytotoxicity of methazole. I. Measurements of species response, spray retention and leaf surface characteristics. *Weed Res.*, **21**, 243-253.

Vermaas, W.F.J. and Govindjee (1981). The acceptor side of photosystem II in photosynthesis. *Photochem. Photobiol.*, **34**, 775-793.

Voss, M., Renger, G., Kötter, C. and Gräber, P. (1984). Fluorometric detection of photosystem II herbicide penetration and detoxification in whole leaves. *Weed Sci.*, **32**, 675-680.

Wain, R.L. and Smith, M.S. (1976). Selectivity in relation to metabolism. In *"Herbicides: Physiology, Biochemistry, Ecology"*, vol. 2, (ed. Audus, L.J.), 279-302, Academic Press, London.

Walker, A. (1971). Effects of soil moisture content on the availability of soil-applied herbicides to plants. *Pestic. Sci.*, **2**, 56-59.

Walker, A. and Featherstone, R.M. (1973). Absorption and translocation of atrazine and linuron by plants with implications concerning linuron selectivity. *J. Exp. Bot.*, **24**, 450-458.

Walker, D.A. (1981). Secondary fluorescence kinetics of spinach leaves in relation to the onset of photosynthetic carbon assimilation. *Planta*, **153**, 273-278.

Wessels,J.S.C. and van der Veen, R. (1956). The action of some derivatives of phenylurethan and of 3-phenyl-1,1-dimethylurea on the Hill reaction. *Biochim. Biophys. Acta*, **19**, 548-549.

Whitehouse, P., Holloway, P.J. and Caseley, J.C. (1982). The epicuticular wax of wild oats in relation to foliar entry of the herbicides diclofop-methyl and difenzoquat. In *"The Plant Cuticle"*, (ed. Cutler, D.F., Alvin, K.L. and Price, C.E.), 315-330, Academic Press, Oxford.

Whybrew, J.E. (1969). Wheat without ploughing. *Agriculture*, **76**, 497-501.

Wiedman, S.J. and Appleby, A.P. (1972). Plant growth stimulation by sub-lethal concentrations of herbicides. *Weed Res.*, **12**, 65-74.

Wills, G.D. and Basler, E. (1971). Environmental effects on absorption and translocation of 2,4,5-T in winged elm. *Weed Sci.*, **19**, 431-434.

Wilson, B.J. and Cussans, G.W. (1975). A study of the population dynamics of *Avena fatua* L. as influenced by strawburning, seed shedding and cultivations. *Weed Res.*, 15, 249-258.

Yamaguchi, S. and Crafts, A.S. (1958). Autoradiographic method for studying absorption and translocation of herbicides using ^{14}C-labelled compounds. *Hilgardia*, **28**, 161-191.

York, A.C. and Arntzen, C.J. (1979). Photosynthetic electron transport inhibition with buthidazole *Abstr. Weed Sci. Soc. Amer.*, 103-105.

Zadoks, J.C., Chang, T.T. and Konzak, C.F. (1974). A decimal code for the growth stages of cereals. *Weed Res.*, **14**, 415-421.

Zweig, G., Tamas, I. and Greenberg, E. (1963). The effect of photosynthesis inhibitors on oxygen evolution and fluorescence of illuminated *Chlorella*. *Biochim. Biophys. Acta*, **66**, 196-205.

11. APPENDIX

11.1 RECIPES

A Soil Types

John Innes No. 2 compost

	% of total
140ℓ sterilised loam	7
60ℓ peat	3
40ℓ Cornish grit	2
1500 g John Innes base	74
280 g ground limestone	14

John Innes base:

Hoof-and-Horn (13% N)	40
Superphosphate (18% P_2O_5)	40
Potassium sulphate (48% K_2O)	20

B. Nutrient Solutions

Long Ashton (Hewitt, 1966).

	mg ℓ^{-1}
KNO_3	20.2
$Ca(NO_3)_2$	65.6
NaH_2PO_4 $2H_2O$	20.8
$MgSO_4$ $7H_2O$	36.9
Micronutrients:	
Ferric citrate	2.45
$MnSO_4 4H_2O$	0.223
$CuSO_4$ $5H_2O$	0.024
$ZnSO_4$ $7H_2O$	0.029

	$mg\ell^{-1}$
H_3BO_3	0.186
$(NH_4)_6Mo_7O_{24}\ 4H_2O$	0.0035
$(Al)_2SO_3\ 12\ H_2O$	0.0186
$Ga(NO_2)_3$	0.05
$CoSO_4\ 7H_2O$	0.0028
$NiSO_4\ 7H_2O$	0.0028

11.2 Summary of analysis of variance

The number labelling each table corresponds to the numerical value used in the results sections.

	Source of variation	df	F	P
Table 2.1	Species	2	13.29	0.001
	Light conditions	2	1.67	NS
	Spp/light	4	0.44	NS
	Error	126		
Figure 2.2	Species	2	11.14	0.001
	Depth	3	95.68	0.001
	Spp/depth	6	2.05	NS
	Error	48		
Figure 2.3	Species	2	859.70	0.001
	Depth	3	65.87	0.001
	Spp/depth	6	51.05	0.001
	Error	48		
Figure 3.3	Species	2	101.00	0.001
	Concn.	1	9.40	0.01
	Light	2	24.60	0.001
	Spp/light	4	5.40	0.001
	Spp/concn.	2	1.80	NS
	Light/concn.	2	0.89	NS
	Spp/light/concn.	4	1.27	NS
	Error	72		
Figure 3.4a	Species	2	28.90	0.001
	Stage	3	6.97	0.001
	Spp/stage	6	2.34	0.05
	Error	48		
Figure 3.4b	Species	2	53.96	0.001
	Stage	3	50.09	0.001
	Spp/stage	6	6.46	0.001
	Error	48		
Figure 3.4c	Species	2	138.70	0.001
	Stage	3	108.60	0.001
	Spp/stage	6	6.40	0.001
	Error	48		
Figure 3.4d	Species	2	98.80	0.001
	Stage	3	259.50	0.001
	Spp/stage	6	19.20	0.001
	Error	48		

	Source of variation	df	F	P
Figure 3.4e	Species	2	0.32	NS
	Stage	3	10.60	0.001
	Spp/stage	6	6.22	0.001
	Error	48		
Figure 3.5a	Species	2	10.60	0.001
	Stage	2	2.57	NS
	Spp/stage	4	2.59	NS
	Error	36		
Figure 3.5b	Species	2	1.22	NS
	Stage	2	2.07	NS
	Spp/stage	4	8.38	0.001
	Error	36		
Figure 3.5c	Species	2	2.27	NS
	Stage	2	2.63	NS
	Spp/stage	4	5.23	0.01
	Error	36		
Figure 3.5d	Species	2	2.71	NS
	Stage	2	8.10	0.001
	Spp/stage	4	4.29	0.05
	Error	36		
Figure 5.2	Species	2	4.70	0.05
	Zone	2	98.10	0.001
	Spp./zone	4	4.20	0.05
	Error	36		
Figure 5.3	Species	2	16.34	0.001
	Depth	2	20.14	0.001
	Spp./depth	4	18.54	0.001
	Error	36		
Figure 5.5	Species	2	34.43	0.001
	Conc.	3	43.86	0.001
	Spp./concn.	6	3.31	0.05
	Error	36		

Aspects of Applied Biology 9, 1985
The biology and control of weeds in cereals

Aspects of the selectivity of barley, *Bromus sterilis* and *Bromus willdenowii* to isoproturon

S. J. HENLY, A. D. DODGE and R. J. STEPHENS
School of Biological Sciences, University of Bath, Bath, Avon. BA2 7AY

Summary. The potential for control of Bromus sterilis and Bromus willdenowii by isoproturon in winter barley has been studied. Although all three species were susceptible when germinated in isoproturon solution, B. willdenowii was most vulnerable. In pot trials at the pre-emergence stage, B. willdenowii was more tolerant than B. sterilis. The greatest selectivity between Bromus species and barley was observed at the first leaf stage. In this and all growth stages, B. sterilis was more sensitive than B. willdenowii. Recovery from photosynthetic inhibition was similar for both Bromus species and much reduced in relation to barley. The varying susceptibility of these species to the action of isoproturon at certain developmental stages is discussed in relation to the possible use of this herbicide.

INTRODUCTION

The rapid proliferation of Bromus sterilis L. (barren brome) as a weed of winter cereal crops over the last decade has been closely associated with the change in farming methods (Ayres & Richardson, 1981). Continuous winter cereal cropping, established by direct drilling or minimum cultivation, provides a suitable environment for this weed to germinate (Pollard, 1982).

B. sterilis is fairly tolerant to many of the herbicides currently used in winter cereals (Froud-Williams, Pollard & Richardson, 1980). The substituted ureas were found to have the greatest effect when used selectively in wheat and barley (Pollard & Richardson, 1981). One of these, isoproturon, showed potential for both pre- and post-emergence control of B. sterilis in pot experiments. However, there are evidently many factors controlling the response of this weed to isoproturon, since there is great variability in the results obtained (Orson, 1981).

In this study, experiments were devised to determine the margin of selectivity of isoproturon between weed and crop, using winter barley as an example of the latter.

Bromus willdenowii Kunth. (prairie grass) was recently introduced into Europe as a forage crop and is currently undergoing trials in Britain (Jordan, 1984). It is possible that B. willdenowii could become a problem weed in cereals if it spreads from neighbouring fields where it is grown as a crop.

Therefore, in view of its biological similarities to B. sterilis and its potential as a weed of cereals, similar studies were undertaken with this species.

The effect of different rates of isoproturon on germination and on plants at various stages of growth in soil was examined. Direct effects of the herbicide on photosynthesis and chlorophyll fluorescence kinetics in seedlings were determined.

MATERIALS AND METHODS

Plant Material. Seeds of B. sterilis were supplied by the Weed Research Organisation, Yarnton, Oxford. They were collected from infested farmland in the Oxford region and stored in hessian sacks at room temperature until required. Seeds of B. willdenowii were obtained from the N.I.A.B. seed handling unit, Cambridge. Treated seeds of winter barley cv. Maris Otter were obtained from seed merchants.

All seeds were pre-germinated in sealed glass trays containing filter paper moistened with distilled water. Seeds of B. sterilis and barley were incubated at 23°C in the dark for 3-4 days and B. willdenowii at 23°C in a 16 h photoperiod at 12 $\mu E/m^2/s^1$ for 5-6 days.

Plant growth conditions. All plants used were grown at either 2 cm depth in John Innes no. 2 compost or in full strength Long Ashton Solution (Hewitt, 1966) in liquid culture beakers in a 'Conviron S-10 h' growth cabinet. A 12 h photoperiod was provided by tungsten bulbs and high output white fluorescent tubes at a photon flux density of 150 $\mu E/m^2/s^1$. Temperatures were maintained at 10/8°C (day/night) and relative humidity at 70/75%.

Germination. The rate and percentage germination of seeds was recorded after 10 days treatment with either 0.5 mM or 1.0 mM isoproturon in Petri-dishes. These were maintained at 23°C in either continuous light, continuous darkness or a 16 h photoperiod.

Uptake. Fresh weights of shoots were measured after 21 days growth in pots containing 2.0 kg a.i/ha isoproturon in either the root or shoot region of the plant (Eshel & Prendeville, 1967).

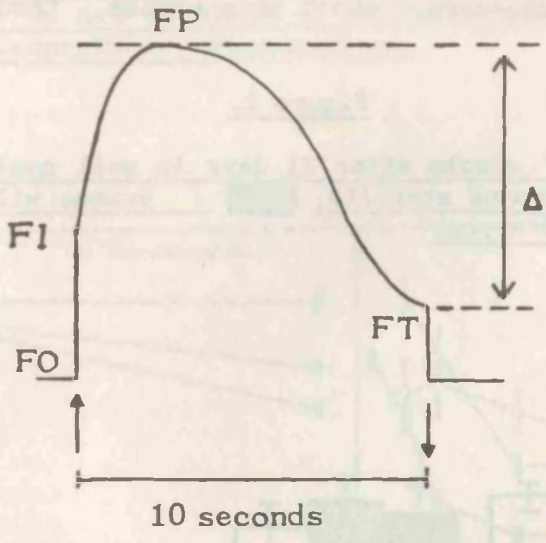
Stages of Growth. Isoproturon was applied at 0.25, 0.75, 1.25, 2.5 and 7.5 kg a.i./ha as a solution in 50 ml distilled water to the soil surface at the various growth stages concerned, namely the pre-emergence, emergence, 1st leaf and 2nd leaf stages (Zadoks, Chang and Konzak, 1974). Fresh weights of shoots were measured 10 days after treatment.

Photosynthesis. Two week old plants grown in liquid culture were treated with 0.1 mM isoproturon for 24 h, then rinsed and Long Ashton solution replaced. Rates of photosynthesis in 0.1 g samples of the 1st and 2nd leaves to emerge were recorded on an Infra-red series 225 gas analyser (Analytical Devp. Co. Ltd., Hoddesdon, U.K.), every 24 h for 7 days following treatment.

Fluorescence. The plants were treated as above. Chlorophyll fluorescence of the middle section of the 1st leaf to emerge was recorded on a plant productivity fluorometer model SF-10 (Richard Branckner Research Ltd., Ottawa, Canada) every 24 h for 7 days after treatment, following a 1 h dark-adaptation period. Results were expressed as ΔF (see Fig. 1), which represents the difference in fluorescence yield between the peak (FP) and 10 seconds (FT) after a dark-light transient (Fedtke & Schmidt, 1983). ΔF is increasingly lowered in plants with inhibited photosynthesis since in treated plants, electron flow through photosystems II to I is curtailed by the herbicide (Dodge, 1983).

Figure 1.

Hypothetical curve to calculate ΔF.



Statistical Analysis. Standard errors of the mean, collected from five replicate treatments, are presented either in parentheses beside each figure or as a bar on line diagrams.

RESULTS

In preliminary tests, B. sterilis seeds exhibited poor germination in continuous light. In all three species, continuous light conditions also increased seed vulnerability to the herbicide (Table 1). Seeds of B. willdenowii were most susceptible to isoproturon, and those that did germinate generally had weak, short roots and poorly developed shoots.

Table 1.

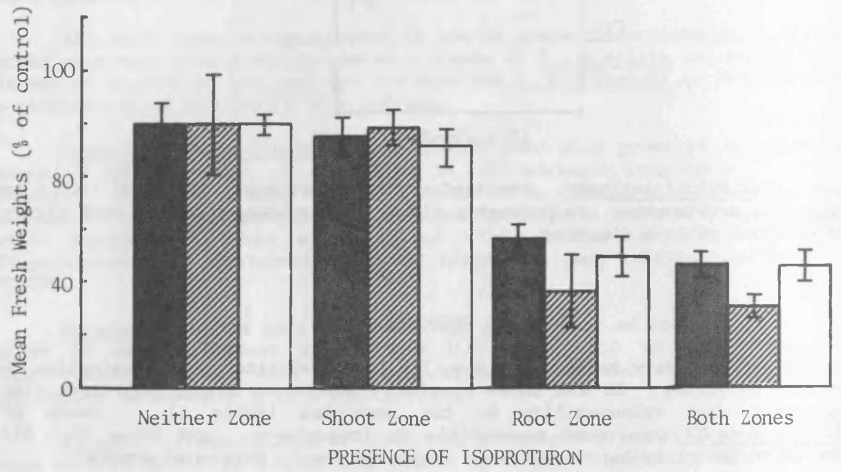
The percentage germination of seeds of Bromus sterilis, Bromus willdenowii and barley after 10 days in a) continuous light, b) continuous darkness and c) in a 16 hour photoperiod at 23°C in isoproturon, expressed as a percentage of the control

	Concentration (mM)	B. sterilis	B. willdenowii	Barley
a)	0.5	71.1 (14.3)	19.5 (8.4)	64.7 (18.2)
	1.0	27.6 (7.7)	19.5 (5.6)	54.9 (19.3)
b)	0.5	95.9 (2.4)	23.3 (6.5)	98.8 (10.2)
	1.0	93.8 (3.6)	20.5 (2.6)	88.8 (15.1)
c)	0.5	94.3 (5.2)	34.8 (10.6)	114.5 (31.7)
	1.0	73.6 (8.6)	23.2 (5.2)	104.8 (30.7)

The pattern of uptake of isoproturon from soil was similar for all three plant species, though slightly greater amounts were absorbed by B. willdenowii (Fig. 2). The presence of isoproturon in both zones of the pot caused the largest shoot fresh weight reductions. Root uptake proved to be more phytotoxic than sub-surface shoot zone uptake, though both contributed to herbicidal damage.

Figure 2.

Reductions in growth of shoots after 21 days in soil containing 2Kg a.i./ ha isoproturon. ■ : Bromus sterilis, ▨ : Bromus willdenowii and □ : barley. I = ± standard error.



In pot trials, B. willdenowii was less susceptible to isoproturon than B. sterilis at every growth stage (Fig. 3). Pre-emergence applications did not sufficiently reduce fresh weights, however application after this stage demonstrated crop and weed selectivity. The most effective reduction in fresh weight of B. sterilis plants resulted from treatment at the 1st leaf stage (Fig. 3c). At this stage, there was a substantial difference between the response of the three species, especially within the range of 1.25 to 2.5 Kg a.i./ha isoproturon. There was little distinction between fresh weights of plants treated with 2.5 and 7.5 Kg a.i./ha, obviating the need to apply such high rates as the latter.

Figure 3.

Shoot fresh weights of Bromus sterilis (●——●), Bromus willdenowii (○——○) and barley (▲——▲) as a % of those of untreated plants, following 10 days growth in various isoproturon concentrations.

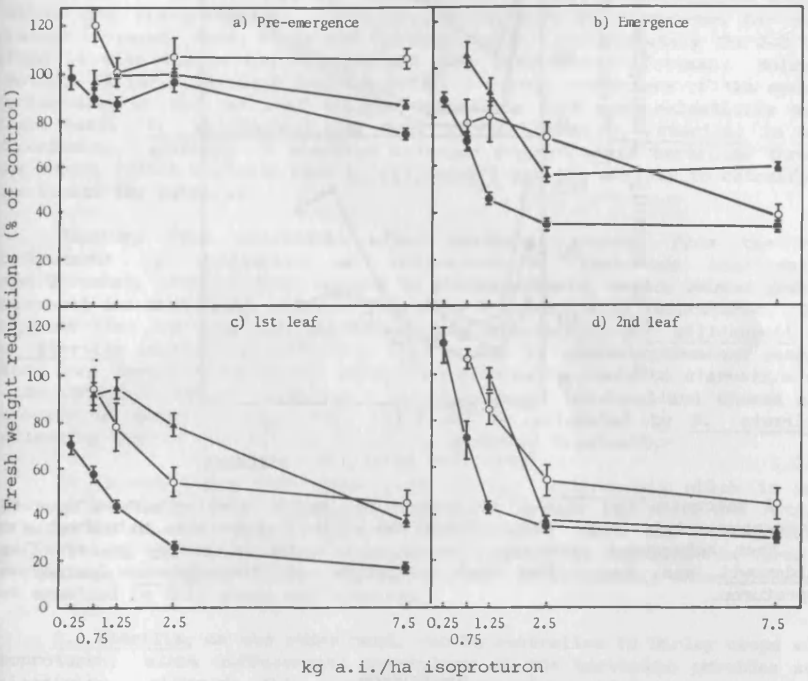
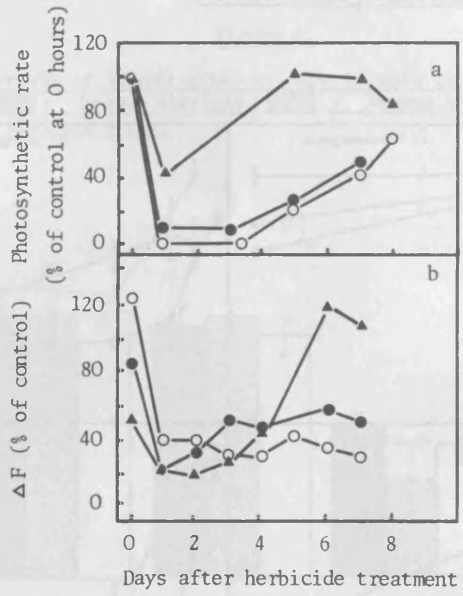


Fig. 4a shows the effect of isoproturon on the photosynthetic rates of the three species. A concentration of 0.1 mM inhibited the rate by as much as 100, 91.5 and 56.3% in B. willdenowii, B. sterilis and barley, respectively, after 24 hours. Removal of the herbicide from the root environment resulted in increased rates of photosynthesis, the recovery rate being related to the degree of inhibition. All three species showed partial recovery, though only barley completely recovered its original rate within the 7 day period. B. sterilis plants died at concentrations above 0.5 mM, but B. willdenowii survived stronger concentrations (data not shown).

Figure 4.

a) Photosynthetic rates and b) fluorescence measurements of Bromus sterilis (●——●), Bromus willdenowii (○——○) and barley (▲——▲) after 24 hours exposure to 0.1 mM isoproturon.



A concentration of 0.1 mM isoproturon had a similar effect upon the fluorescence yield (Fig. 4b). There was an initial decline in the value of ΔF, then subsequent recovery, being most rapid in barley plants. B. willdenowii was again the most sensitive of the Bromus species to isoproturon.

DISCUSSION

The precise mechanism for the selectivity of isoproturon between crop and weed has not been clarified but could involve differences in uptake, retention, translocation and metabolism of the herbicide within the plant.

Photoinhibition in seeds of B. sterilis has been recognised for some time (Froud-Williams, 1981; Pollard, 1982; Hilton, 1982), this enforced dormancy being unique among dark-germinating seeds. Germination percentage of B. willdenowii and barley seeds was reduced in continuous light. This light treatment also promoted herbicidal toxicity in all three species. There seems to be some degree of selectivity at the seed stage, since B. willdenowii was considerably more susceptible to isoproturon in germination tests.

However, in pot trials at the pre-emergence stage, B. willdenowii was more tolerant than both barley and B. sterilis (Fig. 3a). This may relate to herbicide placement in the soil. The great variations in plant response when

a herbicide is applied to the soil may reflect upon the differences in the amounts available to the plant (Walker, 1971). The main route of uptake in all three grasses appeared in these experiments to be via the root system below seed level, which is in agreement with findings by Blair (1978) for other grasses. Therefore differential uptake did not account for selectivity.

The stage of growth of plants has often been shown to have a marked effect upon susceptibility to herbicides. The timing of application in relation to the stage of weed and crop growth is critically important for efficient weed control (Fryer & Makepeace, 1977). The performance of isoproturon was greatly improved when applied at early post-emergence stages, rather than pre-emergence. Similar results have been reported for other grasses (Cussans, Moss, Hance and Embling, 1982). Unfortunately the 2-3 leaf stage is also the period of greatest crop sensitivity (Tottman, Holroyd, Lupton, Oliver, Barnes & Tysoe, 1975). Growth reductions of the species varied most at the 1st leaf stage, suggesting that most selectivity takes place here. B. willdenowii was more tolerant than B. sterilis in soil experiments, although it absorbed a larger proportion of herbicide through the roots. This suggests that B. willdenowii has the ability to detoxify or inactivate the herbicide.

Recovery from inhibition after herbicide removal from the root environment is considered an indication of herbicide inactivation (van Oorschot, 1968). With respect to photosynthesis, barley showed greater tolerance and more rapid recovery following treatment with isoproturon. This suggests that the crop can metabolise the herbicide. B. willdenowii and B. sterilis plants cannot recover fast enough to prevent permanent damage, since long term photosynthetic inhibition eventually leads to starvation and death (Dodge, 1982). However, B. willdenowii survived and showed slow recovery at concentrations that could not be tolerated by B. sterilis, indicating that it has the capability to withstand treatment.

It is not clear from this study why B. willdenowii which is more susceptible at the seed stage, absorbs more through its roots and retains more herbicide per unit weight of leaf tissue, yet can be virtually unaffected by applications to the soil. It cannot be explained in terms of biochemical mechanisms such as photosynthesis, so presumably other processes not examined in this study are involved.

B. sterilis, on the other hand, can be controlled in barley crops with isoproturon, since differential metabolism of the herbicide provides some selectivity, although this has not always been sufficient to provide commercially acceptable control under field conditions.

ACKNOWLEDGEMENTS

The authors are grateful to May & Baker, Ongar, Essex for the gift of isoproturon, and to W.R.O., Oxford, for seed supplies. The work was supported by a MAFF postgraduate studentship to S.J. Henly.

REFERENCES

AYRES, P. & RICHARDSON, W.G. (1981). Some pot experiments to investigate potential control of Bromus sterilis by various herbicides. In Conference Proceedings - Grass weeds in cereals in the U.K., pp. 265-272, Association of Applied Biologists, Wellesbourne, U.K

BLAIR, A.M. (1978). Some studies on the sites of uptake of isoproturon, chlortoluron and metoxuron by wheat, Avena fatua and Alopecurus myosuroides. Weed Research, 18, 381-387.

CUSSANS, G.W., MOSS, S.R., HANCE, R.J. & EMBLING, S.J. (1982). The effect of tillage method and soil factors on the performance of chlortoluron and isoproturon. British Crop Protection Conference - Weeds, 1, 153-160, British Crop Protection Council, Croydon, U.K.

DODGE, A.D. (1982). The role of light and oxygen in the action of photosynthetic inhibitor herbicides. In Biochemical responses induced by herbicides pp. 57-77. (Ed. D.E. Moreland, J.B. St. John and F.D. Hess). American Chemical Society Symposia Series 181, American Chemical Society, USA.

DODGE, A.D. (1983). The mode of action of herbicides. In Progress in Pesticide Biochemistry and Toxicology vol. 3. pp. 163-197. (Ed. D.H. Hutson and T.R. Roberts). John Wiley & Sons Ltd., London, U.K.

ESHEL, Y. & PRENDEVILLE, G.N. (1967). A technique for studying root vs. shoot uptake of soil-applied herbicides. Weed Research, 7, 242-245.

FEDTKE, C. and SCHMIDT, R.R. (1983). Behaviour of metribuzin in tolerant and susceptible soybean varieties. In Pesticide Chemistry - Human welfare and the environment. Vol. 3 pp. 177-182. (Ed. J. Miyamoto). Pergammon, Oxford, U.K.

FROUD-WILLIAMS, R.J. (1981). Germination behaviour of Bromus species and Alopecurus myosuroides. In Conference Proceedings - Grass weeds in cereals in the U.K. pp. 31-40. Association of Applied Biologists, Wellesbourne, U.K.

FROUD-WILLIAMS, R.J., POLLARD, F. & RICHARDSON, W.G. (1980). Barren brome- a threat to winter cereals? In Agricultural Research Council - Weed Research Organisation 8th biennial report, pp. 43-51. ARC, WRO, England.

FRYER, J.D. & MAKEPEACE, R.J. (1977). The principles of weed control in cereals. In Weed Control Handbook vol. 1 - Principles. pp. 261-281. Blackwell, London.

HEWITT, E.J. (1966). Sand and water culture methods used in the study of plant nutrition. In Commonwealth Bureau of Horticulture & Plantation Crops Technical Bulletin No. 22., Commonweath Agricultural Bureau, London, U.K.

HILTON, J.R. (1982). An unusual effect of the far-red absorbing form of phytochrome: Photoinhibition of seed germination in Bromus sterilis L. Planta, 155, 524-528.

JORDAN, W. (1984). New brome grasses for Forage. Information from N.I.A.B., Cambridge. Herbage 04/11.

OORSCHOT, J.L.P. van (1968). The recovery from inhibition of photosynthesis by root-applied herbicides as an indication of herbicide inactivation. British Crop Protection Conference - Weeds, 2, 624-632, British Crop Protection Council, Croydon, U.K.

ORSON, J.H. (1981). The control of Bromus sterilis in cereals with herbicides. ADAS results 1979-1980. In Conference Proceedings - Grass weeds in cereals in the U.K. pp. 291-298. Association of Applied Biologists, Wellesbourne, U.K.

POLLARD, F. (1982). Light induced dormancy in Bromus sterilis. Journal of Applied Ecology, 19, 563-568.

POLLARD, F. & RICHARDSON, W.G. (1981). Chemical control of Bromus sterilis in winter wheat & barley. In Conference Proceedings - Grass weeds in cereals in the U.K. pp. 273-281. Association of Applied Biologists, Wellesbourne, U.K.

TOTTMAN, D.R., HOLROYD, J., LUPTON, F.G.H., OLIVER, R.H., BARNES, T.R. & TYSOE, R.H. (1975). The tolerance of chlortoluron and isoproturon by varieties of winter wheat. In Proceedings European Weed Research Society Symposium - Status & Control of grass weeds in Europe. pp. 360-368.

WALKER, A. (1971). Effects of soil moisture content on the availability of soil-applied herbicides to plants. Pesticide Science, 2, 56-59.

ZADOKS, J.C., CHANG, T.T. & KONZAK, C.F. (1974). A decimal code for the growth stages of cereals. Weed Research, 14, 415-421.